IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

IN RE: '318 PATENT INFRINGEMENT LITIGATION	)	**REDACTED PUBLIC VERSION**
	)	Civil Action No. 05-356-SLR
	)	(consolidated)

PLAINTIFFS' POST-TRIAL ANSWERING BRIEF

APPENDIX III:
TRIAL EXHIBITS AND ADDITIONAL AUTHORITIES

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TABLE OF CONTENTS

APPENDIX I:
TRIAL TRANSCRIPT EXCERPTS

APPENDIX II:
TRIAL EXHIBITS

TAB	PX/DX	EXHIBIT NUMBER
20	PX	631
21	PX	632
22	PX	633
23	PX	653
24	PX	663
25	PX	680
26	PX	704
27	PX	706
28	PX	714
29	PX	719

APPENDIX III:
TRIAL EXHIBITS AND ADDITIONAL AUTHORITIES

TAB	PX/DX	EXHIBIT NUMBER
30	PX	727
31	PX	756
32	PX	763
33	PX	810
34	PX	811
35	PX	812
36	PX	818
37	PX	820
38	PX	829
39	PX	833
40	PX	1061
41	PX	1122
42	PX	1181
43	PX	1223
44	PX	1228
45	PX	1245
46	PX	1246
47	PX	1247
48	PX	1248
49	PX	1321
50	PX	1339
51	PX	1350
52	PX	1364
53	PX	1365
54	PX	1366
55	PX	1372
56	PX	1397

TAB	PX/DX	EXHIBIT NUMBER
57	PX	1398
58	PX	1399
59	PX	1400
60	PX	1401
61	PX	1401a
62	PX	1402
63	DX	74
64	DX	483
65	DX	557
66	DX	629
67	DX	651
68	DX	655
69		United States Patent and Trademark Office Memorandum, "Supreme Court decision on *KSR Int'l Co., v. Teleflex, Inc.*", May 3, 2007

EXHIBIT 30

131

VOL. 19, NO. 2, 1983

The Cholinergic Treatment Strategy in Aging and Senile Dementia[1]

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Introduction

Cognitive deterioration is an almost universal phenomenon in the geriatric population. The majority of healthy elderly people experience "benign senescent forgetfulness," a mild loss of memory for details of recent events (Kral 1978). A substantial subgroup are more severely afflicted, and 3 to 4 percent of the population of the United States above 65 develop senile dementia of the Alzheimer's type (SDAT) (Katzman 1976; Plum 1979), in which memory impairment progresses to a state of profound cognitive disturbance wherein self-care is impossible, followed by a significantly premature death. While at present neither the slow cognitive decline of the elderly nor the accelerated deterioration of SDAT patients can be arrested or reversed, current studies to elucidate underlying histologic and biochemical pathology may indicate rational treatment approaches.

Multiple central nervous system (CNS) dysfunctions have been identified in SDAT, including changes in the concentration of norepinephrine, serotonin, dopamine, and various neuropeptides, but by far the most consistent finding is a large reduction of choline acetyl transferase (CAT) activity, an enzyme localized in cholinergic neurons. A large body of evidence suggests that this disruption of the cholinergic system is a major factor in cognitive impairment in SDAT and may also play a role in more benign age-related memory loss. This "cholinergic hypothesis" has important practical as well as theoretical implications, since a logical deduction is that pharmacologic enhancement of the cholinergic system in aged and SDAT patients should improve their cognitive functioning. Trials of pharmacologic agents believed to enhance central cholinergic activity have been carried out in memory-impaired (mainly SDAT) patients.

While learning and memory impairment may ultimately prove to be due to a complex interaction of noncholinergic as well as cholinergic neurotransmitter system abnormalities, a discussion of the as of yet inconclusive body of data pertaining to noncholinergic CNS deficits is beyond the scope of this paper. We have therefore confined this review to investigations of the role of cholinergic deficits in cognitive impairment, focusing particularly on studies of its clinical exploitability, in order to identify the current state of knowledge, to clarify areas of controversy, and to suggest future avenues of investigation.

Neurochemical and Histologic Evidence for the Cholinergic Hypothesis

The hippocampus and frontal cortex, areas of the brain rich in cholinergic neurons, are thought to be involved in learning and memory. Histologic validation of the cholinergic hypothesis therefore requires that a cholinergic deficit be demonstrated in these brain areas in memory-impaired patients. In all nondemented elderly patients, widespread neuronal loss occurs. The mean brain weight of healthy people begins to decrease after age 50 (de Kaban & Sadowsky 1978), and histologic studies

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Plaintiff's Exhibit
PX - 727

show a steady loss of cerebral cortical neurons (Brody 1955) and Purkinje cells (Corsellis 1976), and a linear decrease of hippocampal cortical neurons to 80 percent of total hippocampal neuronal density by age 95 (Ball 1977). Neurochemical analyses of central cholinergic activity have not reported large or reliable decreases in CAT activity as a function of aging (Bird & Iverson 1974; Bowen et al. 1976; Carlsson et al. 1980; Davies 1978a; McGeer & McGeer 1975, 1976; Perry et al. 1977; Spillane et al. 1977; Spokes 1979; White et al. 1977). However, a 20 percent decrease of sodium-dependent high-affinity choline uptake under basal conditions has been measured in the hippocampus of aged rats, which normalized with K^+ stimulation (Sherman et al. 1981). Other studies in mice confirm an age-related decrease of acetylcholine (ACh) synthesis *in vivo* (Gibson et al. 1981) but not *in vitro* (Sherman et al. 1981; Sims et al. 1982). Studies of postsynaptic cholinergic receptors are more conclusive: five of six rodent studies (Freund 1980; James & Kanungo 1976; Lippa et al. 1980, 1981; Morin & Wasterlaine 1980; Strong et al. 1980) and two of three aged human studies (Davies & Verth 1978; Perry 1980; White et al. 1977) have replicated a decrease of cortical postsynaptic muscarinic receptor binding. As receptor affinity is unchanged, these studies appear to reflect a decreased density of postsynaptic muscarinic receptors with aging. Taken collectively, these neurophysiologic data suggest that some changes in the cholinergic neuronal system occur with age. These changes, however, are not large, either absolutely or when compared to changes seen in other neurotransmitters (such as catecholamines) with age, and their functional significance is not clear. At the least, they may suggest that elderly people lose a substantial degree of neuronal redundancy, which may make them more susceptible to neuronal dysfunction than younger people with their full complement of healthy nerve cells.

The principal histologic hallmarks of SDAT, neurofibrillary tangles and neuritic plaques are concentrated in the hippocampus and cortex (Jervis 1971) and correlate with severity of dementia (Blessed et al. 1968; Wilcock & Esiri 1982). CAT activity is consistently and markedly decreased in these same brain areas (Bartus et al. 1982) and is quantitatively correlated both to plaque number and to severity of dementia (Perry et al. 1978), as measured by cognitive testing scales such as the Memory and Information Test (MIT) (Roth & Hopkins 1953) or the Dementia Rating Scale (DRS) (Blessed et al. 1968). This decrease of CAT activity is likely to be due, in part, to selective degeneration of presynaptic cholinergic neurons which originate in the nucleus basalis of Maynert (nbM). Large acetylcholinesterase-rich neuronal bodies in the nbM project widely to the cerebral cortex, and similar neurons in the adjacent diagonal band of Broca (dbB) and medial septum project to the hippocampus (Mesulam et al. 1982), providing the major source of extrinsic cholinergic input to these areas. A decrease of up to 80 percent of these neurons has been demonstrated in the nbM of SDAT patients (Price et al. 1982; Whitehouse et al. 1981, 1982; Wilcock et al. in press). Degenerating neuronal axons in the cortex that originate in the nbM may provide the substrate for senile plaques; evidence for this has come from histologic studies of evolving plaques which reveal that immature plaques contain acetylcholinesterase-rich dystrophic axons which disappear as the plaques condense with age (Struble et al. 1982). Taken together, the histologic data strongly suggest that a selective loss of cholinergic neurons from the nbM is a major, specific pathologic mechanism in SDAT.

SDAT brains do not differ from age-matched controls in their ability to bind muscarinic antagonists in the cortex (Antuono 1980; Bowen & Davison 1980; Davies 1978b; Davies & Verth 1978; Nordberg et al. 1980; Perry 1978, 1980; Perry et al. 1977; Reisine et al. 1978; White et al. 1977), suggesting that postsynaptic muscarinic cholinergic receptors are not affected in SDAT. This does not mean that SDAT patients have a "normal" density of muscarinic receptors, but rather that decrements of receptor density are no greater in SDAT than in normal aged brains.

In-vivo confirmation of a selective cholinergic deficit in SDAT might be obtained by cerebrospinal fluid (CSF) analysis. This has been hindered by the technical difficulties of assaying minute quantities of ACh. Gas chromatog-

raphy/mass spectrometry (GCMS) now appears to be a reliable technique for measuring ACh and choline *in vivo* (Jenden et al. 1973), and these measures as well as homovanillic acid, 5-hydroxyindoleacetic acid (5-HIAA), and 3-methoxy-5-hydroxyphenyl-ethyleneglycol (MHPG) were obtained in 10 male and 4 female SDAT patients (Davis et al. 1982). When correlated with severity of cognitive impairment as measured by the MIT score, in which a lower score indicates increased severity of dementia, the results confirmed a selective ACh deficit in direct proportion to clinical cognitive impairment: Patients with greater memory impairment tended to have lower CSF ACh (Pearson product-moment correlation coefficient of MIT with ACh $r = 0.76$, $p<.005$). There was a nonsignificant trend for higher choline levels to be associated with greater severity of dementia, and the ratio of choline to ACh was inversely correlated to MIT score ($r = -0.79$, $p<0.001$). There was no significant correlation between severity of dementia and levels of other neurotransmitter metabolites measured. Thus the marked cholinergic deficits suggested by postmortem studies seem also to be reflected in the CSF of patients with SDAT; the close correlation of CSF-ACh and cognitive testing scores lends further credence to the hypothesis that the cholinergic deficit is functionally significant.

Repeated demonstration of a specific marked cholinergic deficit in SDAT suggests but does not confirm that dysfunction of this neurotransmitter system is related to learning and memory impairment. The relationship is even more tenuous in normal aging, where perturbations of many neurotransmitter systems to a degree that is equal to or greater than cholinergic abnormalities have been reported. If losses of cholinergic function contribute to memory impairment, pharmacologic disruption of the cholinergic system in young healthy adults should produce cognitive disturbances that mimic those seen in old age and SDAT. Administration of the antimuscarinic agent scopolamine to young normal subjects does in fact produce transient deficits in memory for recent but not immediate events, deficits similar to those found in naturally aged sugjects (Drachman & Leavitt 1974). These effects in young subjects are reversed by physostigmine, an acetylcholinesterase inhibitor, but not by amphetamines (Drachman 1977), specifically implicating the anticholinergic action of scopolamine in impairing cognition rather than its more general sedating effects which impair attention and alertness. Similarly, scopolamine-induced impairment of task performance requiring memory for recent sensory events in young monkeys paralleled consistent deficits in aged monkeys (Bartus & Johnson 1976). Again, the effects of scopolamine were partially reduced by physostigmine (Bartus 1978). Pharmacologic agents that block other neurotransmitters did not reproduce naturally occurring deficits (Bartus 1980). Conversely, memory enhancement in young normal subjects follows administration both of physostigmine and arecoline, a muscarinic agonist. The component of memory that is specifically enhanced by these agents is encoding of new information beyond immediate memory (i.e., long-term memory [LTM]); they have no effect on short-term memory (STM), or on retrieval of information from LTM (Davis et al. 1978; Sitaram et al. 1978), a pattern that is typical of SDAT (Torak 1978). Diminished cholinergic activity is therefore implicated in the etiology of memory loss in aging and SDAT.

Cholinergic Treatment Strategies

Evidence that the cholinergic deficit seen in SDAT correlates with histopathology and clinical symptomatology and that a pharmacologically induced cholinergic deficit produces cognitive impairments that mimic those seen in aging and SDAT, provides strong support for the hypothesis that cholinergic dysfunction has a central role in the etiology of age-related and SDAT memory deficits. Since the clinical importance of the cholinergic hypothesis lies in its suggestion of a rational treatment approach, many investigators are attempting to facilitate memory via enhancement of cholinergic activity. Pharmacologic agents that may produce such enhancement can be grouped into three categories: presynaptic agents that might increase ACh production and release, synaptic agents that increase available ACh by limiting its breakdown, and postsynaptic agents that directly stimulate ACh receptors.

Trials of the ACh precursors choline and lecithin in memory-disordered patients were encouraged by findings that large amounts of these precursors can, under some conditions, induce increases in ACh concentration (Cohen & Wurtman 1975; Haubrich et al. 1974). Results to date are predominantly negative; 15 studies in SDAT (Boyd et al. 1977; Brinkman et al. 1982a, 1982b; Christie et al. 1979; Etienne et al. 1978a, 1978b, 1981; Fovall et al. 1980; Peters & Levin 1979; Renvoize & Jerram 1979; Signoret et al. 1978; Smith et al. 1978; Thal et al. 1981; Vroulis et al. 1981; Whitely et al. 1973) and 3 in normal elderly (Ferris et al. 1979; Mohs et al. 1979, 1980) in which either choline or lecithin was given in many dosages over time periods ranging from 1 day to 3 months yielded, with one exception, no significant cognitive improvement. These trials may have failed because the validity of the assumption on which they are based, namely, that peripherally-administered precursors enhance central cholinergic activity, remains controversial. ACh synthesis in cholinergic nerve terminals appears to be directly linked to choline uptake via a sodium-dependent high-affinity transport system which is almost completely saturated under normal conditions (Haga & Noda 1973; Yamamura & Snyder 1973). Increasing the level of available choline has not been shown to have any direct effect on spontaneous release of ACh (Bierkamper & Goldberg 1979). Choline pretreatment does, however, appear to facilitate ACh synthesis under conditions in which there is an increase in the firing rate of these neurons. Increased firing of presynaptic cholinergic neurons induced either indirectly by atropine administration (Wecker et al. 1978) or directly by electrical stimulation (Millington & Goldberg 1981) normally depletes ACh, but this effect can be reversed by pretreatment with choline. Since under normal conditions the completely saturated high-affinity uptake system is rate-limiting, the proposed mechanism for this pretreatment effect is that under conditions of increased neuronal demand a low-affinity uptake system transports the extra choline needed for ACh synthesis (Goldberg 1982). This suggests that exogenously-administered choline or lecithin might be of use when agents that enhance the firing rate of presynaptic neurons are administered simultaneously. To date, no pharmacologic agents have convincingly demonstrated such an effect on firing rate, although piracetam has attracted interest as a potential enhancer of neuronal metabolic activity. While its precise mechanism of action is unclear, it has been shown to improve CNS functioning under hypoxic conditions (Nickolson & Wolthius 1976) and to reduce hippocampal ACh levels, which may be secondary to a piracetam-mediated acceleration of ACh release (Wurtman et al. 1981). While further preclinical work delineating the precise mechanism of action of piracetam is needed before a full understanding of its clinical potential can be gained, this combination given to aged rats improved retention of a passive avoidance task which neither agent given alone duplicated (Bartus et al. 1981). Furthermore, three of ten SDAT patients given the same combination for 1 week showed significant improvement in cognitive testing (Friedman et al. 1981). These pilot data suggest that a subgroup of SDAT patients may be helped by piracetam and precursor, and encourages further trials. Other agents that may have potential for enhancing neuronal utilization of exogenously administered precursors are guanidine and 4-aminopyridine. These drugs stimulate presynaptic release of ACh and may also stimulate its synthesis; their mechanism of action is thought to be facilitation of calcium influx which increases the amount of transmitter released per nerve impulse (Lundhand & Thesleff 1977). Both agents have been safely administered to humans, and 4-aminopyridine is believed to cross the blood-brain barrier freely. Guanidine, used to enhance peripheral cholinergic activity in Eaton-Lambert Syndrome, does not normally cross the blood-brain barrier. However, this barrier may not be intact in SDAT (Wisniewski & Kozlowski 1982), providing a theoretic rationale for testing this agent as well as 4-aminopyridine in conjunction with ACh precursors in SDAT.

Even if ACh synthesis could be significantly increased by pharmacologic manipulation, consideration of the pathologic processes in SDAT leads one to conclude that any precursor therapy might have only limited usefulness

in reversing cognitive impairment. SDAT is characterized by an extensive loss of cholinergic neurons, yet precursor therapies require cholinergic neurons that are functionally intact and capable of increasing ACh synthesis to compensate for the loss of surrounding neurons. This may not be possible to any clinically meaningful extent. Alternative treatment strategies involving attempts to increase available ACh by blocking its degradation at the synapse circumvent some of the problems inherent in precursor strategies. The only currently available relatively safe pharmacologic agents of this type are physostigmine, which produces chronic enhancement of the cholinergic system by competitively inhibiting acetylcholinesterase, and tetrahydroaminoacridine (THA), a centrally-acting reversible acetylcholinesterase inhibitor with a longer half-life than physostigmine. Of these, parenteral physostigmine has been the most widely studied in patients with SDAT and in normal elderly, with promising results. However, the variability of these results, both in terms of overall efficacy and specific areas of improvement, has generated debate over the clinical utility of cholinesterase inhibitor therapies. The apparently contradictory nature of some findings may be explained and reconciled by careful review of appropriate study design and patient selection.

Studies of cholinesterase inhibitors are methodologically not straightforward. Animal and human studies have shown that intravenously administered physostigmine has an extremely narrow therapeutic window, and optimal doses vary from individual to individual. In aged monkeys, reliable and replicable facilitation of memory occurs during intravenous physostigmine administration, but the optimal dose varies dramatically—from 0.005 mg/kg to 0.05 mg/kg—between subjects (Bartus 1979). Doses of physostigmine that exceed the therapeutic window may in fact worsen performance on cognitive tests, as was demonstrated in healthy young human volunteers who received intravenous physostigmine in doses exceeding 1.5-2.0 mg (Davis et al. 1976). Therefore, an adequate test of physostigmine's efficacy requires that multiple doses be administered to each patient to establish individual "best doses." Awareness of this narrow and individualized dosage requirement has led to an investigational strategy employing a novel two-phase investigational method (Davis & Mohs 1982). Patients are first given various doses of physostigmine and placebo in a random, double-blind fashion and are tested to determine a best dose. After the "dose-finding" phase, the dose of physostigmine associated with the subject's best performance is repeated as is a placebo dose, again randomly and double-blind, to test the replicability of the first-phase response.

Nonuniformity of tasks devised to measure learning and cognition may also account for variability of results. An appropriate task would be specific, that is, would test one particular cognitive skill such as encoding into LTM that is impaired by aging or SDAT and that is hypothetically sensitive to fluctuations in cholinergic activity. It would have to be comprehensible to the subject but of sufficient difficulty to allow room for improvement as well as deterioration. Alternative forms of the task should be readily constructed for repeated testing, and it should be short enough to be completed while the experientally-administered drug is at a steady-state blood level. It follows that to accurately assess effects of physostigmine, tests would have to be individually modified according to the severity of the patient's dementia. In nondemented people, one of the tests that is most sensitive to cholinergic drug effects is a word recall task in which subjects are asked to learn a long list of words that exceeds their immediate memory span, hence requiring storage of the items into LTM. Such a task is often incomprehensible to SDAT patients. Alternative strategies to measure storage into and retrieval from LTM have therefore evolved, such as the development of a Recognition Memory Task (RMT) (Mohs et al. 1982). The test consists of showing patients 12 words or pictures which they are asked to describe or read. These are then shown to the patient again intermixed with 12 unfamiliar words or pictures and the patient is asked whether or not he or she recognizes each item. More severely demented patients are shown pictures and less severely demented patients are tested with words; for every patient with mild to moder-

ately severe dementia, one of the two tests is both easy enough so that the patient could demonstrate some learning yet difficult enough so that the patient could demonstrate improvement. Thus, accurate determination of physostigmine's effects on long-term memory may depend to some extent on the choice of appropriate and comprehensible assessment instruments.

Characteristic cognitive and behavioral impairments in SDAT are not limited to LTM, and there are suggestions that physostigmine might be effective in partially reversing several of these deficits. Some therapeutic actions might go unnoticed unless tasks that appropriately measure such deficits are utilized. Only one study (Muramoto et al. 1979), for example, tested constructional abilities via a drawing task and found a marked, reproducible improvement. Likewise, the one study that examined inappropriately recalled information ("intrusion errors") saw a significant decrease of such errors during physostigmine administration (Smith & Swash 1979). These errors are significantly associated with decreased CAT activity and increased numbers of senile plaques on autopsy and appear to occur significantly more frequently in SDAT patients (Fuld et al. 1982) than in patients with dementias of other etiologies, suggesting that they occur as a consequence of decreased cholinergic activity. A similar phenomenon was discovered in the one study (Davis et al. 1982) which analyzed the number of false positive responses given by each SDAT patient in a recognition task per testing day and found a significant decrease of these errors on physostigmine days. Signal detectability analysis of the data indicates that physostigmine increased the discriminability of old from new items (Mohs & Davis 1982), possibly because more information about the old items had been learned and stored in memory, making old items more familiar to the patients. The length of testing batteries is, of course, limited both by the patient's attention span and by the brief half-life of physostigmine; however, careful selection of tasks that measure these and other cognitive and behavioral faculties that are suspected to be cholinergically sensitive might elucidate whether cholinomimetic therapy has multiple salutary effects in SDAT.

Patient selection is another variable that may influence the outcome of physostigmine trials. While most studies of physostigmine in SDAT outline clinical criteria used to eliminate patients with dementias of other etiologies, few used stringent criteria either to increase the likelihood that their parents did in fact have SDAT or to objectively rate the severity of each patient's dementia. Long-term studies have consistently shown that diagnostic accuracy in SDAT as confirmed by autopsy can be as low as 65 percent, and is particularly likely to be incorrect in younger patients and those with less severe dementias (Heston & Mastri 1982; Ron et al. 1979). The use of rating scales such as the MIT or DRS, which are significantly correlated with the extent of histopathologic changes seen on autopsy, can improve diagnostic accuracy: scores of 10 or less on the MIT, or 4 or more on the DRS have been shown to select patients with a high probability of having SDAT (Kay 1977). A group of patients who did not meet these criteria could therefore be comprised of a heterogeneous population which includes a high proportion of depressives. These patients would not be expected to respond to cholinomimetics and could in fact worsen when cholinergic agents, which theoretically may exacerbate depression (Davis et al. 1978), are administered. These issues are especially critical when small patient cohorts are being tested.

In summary, the best designed studies of physostigmine in SDAT are those that have a large population of stringently diagnosed patients whose severity of dementia has been rated with autopsy-validated scales. Patients should be tested with several doses of physostigmine to ascertain an individualized optimal dose, and appropriate peripheral cholinergic blockade might be administered to prevent distressing side effects. Testing devices should be chosen to demonstrate alterations of function in several areas and should be individually modified to conform with each patient's capabilities. Those studies that most closely approximate these conditions show that learning and memory are significantly improved by low doses of parenteral physostigmine.

Of the seven studies of parenteral physostigmine in SDAT (Ashford et al. 1981; Chris-

tie et al. 1981; Davis et al. 1982; Muramoto et al. 1979; Peters & Levin 1979; Smith & Swash 1979; Sullivan et al. 1982), five (Christie et al. 1981; Davis et al. 1982; Muramoto et al. 1979; Smith & Swash 1979; Sullivan et al. 1982) demonstrated mild to moderate transient improvements during physostigmine administration. Four investigations medicated SDAT patients with several doses of parenteral physostigmine, and three of these (Christie et al. 1981; Davis et al. 1982; Sullivan et al. 1982) reported significant improvement on some doses of physostigmine; the fourth (Peters & Levin 1979) studied only three patients and found that one improved on physostigmine. Two of the positive studies (Christie et al. 1981; Sullivan et al. 1982) demonstrated improvement on doses lower than 0.5 mg intravenously (i.v.) which were not reproduced when the dose was raised to 0.5 mg or higher, underlining the narrowness of the dose-response window and the care that must be taken to avoid overshooting an optimal dose. It should be noted that these two studies grouped and analyzed data by dose rather than by individual patient performance, which does not completely satisfy the premise that patients should be tested at their own unique "best dose." Only one investigational team employed the two-phase study design outlined previously. In this study (Davis et al. 1982), all but 2 of the 18 patients had their best performance on a memory task on some dose of physostigmine. On replication physostigmine significantly enhanced cognitive performance ($p = 0.02$, paired t-test, two-tailed).

When only one dose of physostigmine is given to patients, one is less likely to see positive results. The three investigations which tested one dose of physostigmine in patients with SDAT used 0.5 mg i.v. (Ashford et al. 1981) or 1 mg subcutaneously (s.q.) (Muramoto et al. 1979; Smith & Swash 1979), which are relatively high doses. One study (Ashford et al. 1981) failed to show any cognitive improvement, although one study reported significant improvement of constructional skills (Muramoto et al. 1979) and another found that "intrusion errors" decreased significantly (Smith & Swash 1979), categories that were not routinely tested in other studies. While these two studies could not demonstrate learning and memory improvement, they each examined only one patient, and in one case (Muramoto et al. 1979) utilized a word-recall task that was noted to be quite difficult for the patient.

The tests that were actually used to assess the effects of physostigmine upon cognition varied from study to study. Three (Ashford et al. 1981; Muramoto et al. 1979; Peters & Levin 1979) of the seven studies of physostigmine in SDAT used recall but not recognition tasks. Since such a test is often too difficult and incomprehensible to have clinical utility in SDAT patients, this choice of assessment scale may in some part account for the fact that none of these studies were able to demonstrate learning or memory improvement with physostigmine. Conversely, three (Christie et al. 1981; Davis et al. 1982; Sullivan et al. 1982) of the four studies that included recognition tasks in their testing batteries established that significant improvement on these tasks did occur with physostigmine. All three of these studies used multiple doses of physostigmine as well, thus approximating the most ideal study design.

Two studies tested the cognitive response of normal elderly to parenteral physostigmine, and were divided about physostigmine's efficacy: One reported a 12 percent improvement of memory storage ($p = 0.06$) (Drachman & Sahakian 1980) and one failed to demonstrate memory changes (Drachman et al. 1982). Both appropriately utilized recall memory tasks but administered only one dose of physostigmine without a comparable placebo day. One of these normal elderly studies (Drachman et al. 1982) and one unsuccessful study of physostigmine in SDAT (Peters & Levin 1979) also experimented with a combination of physostigmine and lecithin; SDAT patients did have significant enhancement of cognition on this combination while normal elderly did not.

Improvements produced by i.v. physostigmine are of theoretical importance, but whether this can be translated into practical pharmacologic intervention in SDAT requires trials of long-acting, safe oral cholinomimetic drugs. Physostigmine given orally has a short half-life, but in multiple small doses it produces chronic enhancement of cholinergic systems. Clinical trials of oral physostigmine have be-

gun recently. These studies are made difficult by the lack of information equating parenteral and oral doses of physostigmine and this, combined with the variability in the effective parenteral dose of physostigmine, makes a variable dosage procedure, similar to the one described for i.v. physostigmine, imperative. In preliminary studies this strategy did yield modest improvements, both in specific cognitive areas and overall functioning, in some SDAT patients (Davis et al. 1982; Thal et al. 1982). Combination therapy of oral physostigmine plus precursor may further improve response, and current investigation of this hypothesis using oral physostigmine and lecithin in patients with early SDAT has shown some success (Thal et al. 1982).

THA, also available in parenteral and oral preparations, has a longer life than oral physostigmine and has few peripheral side effects (Albert & Glendhill 1945), making it an attractive pharmacologic agent. THA has not yet been extensively tested in SDAT patients; only one parenteral (Summers et al. 1981) and one oral trial (Kaye et al. 1982) have been completed to date. Moderately demented but not severely demented patients given intravenous THA improved on orientation questions and global ratings, although specific effects of THA on learning were apparently not measured. Beneficial effects were also seen when oral THA was given in combination with lecithin to mild to moderately impaired SDAT patients, but did not occur when either agent was given alone (Kaye et al. 1982). Again, more severely impaired patients did not respond to any treatment. Tasks selected to assess improvement in this study were all word-recall tasks, which are quite difficult for most SDAT patients. Further trials of oral THA in a wide range of stringently diagnosed SDAT patients utilizing specific and appropriate cognitive testing scales should clarify whether this agent is in fact clinically useful in a subgroup of demented patients.

Acetylcholinesterase inhibitors show more promise as a treatment strategy in SDAT than presynaptic agents, perhaps because they do not require presynaptic neurons to augment the synthesis of acetylcholine. Both strategies, however, share a fundamental limitation in that they are dependent on an intact presynaptic neuron to provide a substrate for their activity. These problems may be circumvented by administering cholinergic agents which work directly at postsynaptic receptor sites, since the number of postsynaptic muscarinic binding sites is not reduced in SDAT patients as compared to age-matched controls. Theoretical difficulties revolve around the uncertainty as to whether these compounds would act in a manner that is physiologically equivalent to the release of Ach from an intact presynaptic neuron. Such agents might flood receptors rather than selectively stimulate neurons. Unusual temporal patterns of receptor activation might also result as the agonist is deactivated. Within a narrow therapeutic range, however, these agents could be promising. Arecoline given to normal young humans and aged nonhuman primates has enhanced learning (Bartus et al. 1980; Sitaram et al. 1978). Significant cognitive improvement was also measured when arecoline was infused intravenously in a small group of patients with presenile dementia (Christie et al. 1981). Unfortunately, arecoline has a half-life that is too short to be clinically useful. Oral pilocarpine given alone or with lecithin was not successful in altering learning and memory although a very small, mixed-diagnosis demented patient cohort was studied (Caine 1980). Oxotremorine, a longer-acting muscarinic agent, may be a more ideal compound, and clinical confirmation of its usefulness in SDAT awaits therapeutic trials.

All cholinergic treatment strategies presuppose the presence of some quantity of functionally intact cholinergic neurons. Since SDAT is a progressive disorder with clinical and neuropathological characteristics which become more severe over time, these treatment strategies should only be effective in those patients who still possess this hypothetical "critical mass" of cholinergic neurons. Such patients might be identifiable on the basis of biologic parameters that predict or correlate with treatment response. While such parameters have yet to be fully defined, one pilot study (Friedman et al. 1981) evaluating the effects of choline and piracetam found that the ratio of red blood cell to plasma choline identified a subgroup of SDAT patients who improved on this drug

combination. Similarly, preliminary work (Davis, B.M. et al. 1982) suggests that neuroendocrine parameters such as overnight cortisol concentration might correlate with a positive response to oral physostigmine. Hence, an exciting area of investigation is the search for antemortem biologic markers that may serve as predictors to pharmacologic response.

The action of neuropeptides in SDAT and aging deserves mention as a future avenue of investigation. Although they are a class of compounds quite different from the cholinergic agents previously discussed, they coexist with and serve to potentiate the action of neurotransmitters in some neurons (Hökfelt et al. 1980) and may be closely tied to the cholinergic system. Decreased somatostatin immunoreactivity, for example, has been seen in the cerebral cortex and hippocampus of SDAT patients (Davies & Terry 1981; Rossor et al. 1980). While the significance of this finding has not been elucidated, another neuropeptide, vasoactive intestinal polypeptide (VIP), is released with ACh in cat submandibular glands and was found to increase ACh-induced salivary secretion via a VIP-induced 10^5-fold enhancement of muscarinic receptor affinity for ACh (Lundberg 1982). However, cortical VIP activity was not found to be diminished in SDAT brains (Rossor et al. 1980). Extrapolation from these data suggest that cholinergic therapy could only be maximally effective when neuropeptide cofactors that may regulate sensitivity to ACh are also administered.

Conclusion

SDAT is a multisymptom disease that may ultimately prove to be mediated by multiple neurochemical abnormalities. Alterations of many neurotransmitter systems, neuropeptides, and receptor sites may contribute to clinical impairment but have yet to be completely elucidated. Despite these qualifications, there is compelling evidence that supports the hypothesis that cognitive impairment in SDAT is based to a large extent on disruption of central cholinergic systems. We have reviewed evidence that demonstrates that a profound cholinergic deficit exists in the cortex and hippocampus of SDAT patients, areas of the brain associated with learning and memory, and that the amount of deficit correlates both with the extent of classic histologic hallmarks of SDAT and with severity of dementia. Furthermore, pharmacologically-induced reduction of cholinergic activity in normals produces cognitive deficits typical of SDAT. Most importantly, for herein lies the potential clinical usefulness of the cholinergic hypothesis, pharmacologically induced enhancement of cholinergic activity has been shown to improve cognitive functioning in SDAT patients. The success of cholinomimetic treatment strategies thus far has been modest, and definitive treatment of SDAT awaits clarification of complex neurochemical variables, delineation of parameters that can predict response, and development of appropriate safe, long-acting pharmacologic agents. Nonetheless, trials of pharmacologic agents that enhance cholinergic activity should be aggressively pursued, as they offer a rational treatment strategy based on observed neurochemical deficits.

References

Albert, A., and Glendhill, W. Amino acridines. *J. Soc. Chem. Ind.*, 64:169, 1945.

Antuono, P., Sorbi, S., Bracco, C., Fusco, T., and Amaducci, L. A discrete sampling technique in senile dementia of the Alzheimer type and alcoholic dementia: Study of the cholinergic system. In: Amaducci, L., Davison, A.N., and Antuono, P. (eds.), *Aging of the Brain and Dementia* (Series: *Aging, Vol. 13*). New York:Raven Press, 1980, pp. 151-158.

Ashford, J.W., Soldinger, S., Schaeffer, J., Cochran, L., and Jarvik, L.F. Physostigmine and its effect on six patients with dementia. *Am. J. Psychiatry*, 138:829-830, 1981.

Ball, M. Neuronal loss, neurofibrillary tangles, and granulovacuolar degeneration in the hippocampus with aging and dementia. *Acta Neuropathol.*, 37:111-118, 1977.

Bartus, R.T. Evidence for a direct cholinergic involvement in the scopolamine-induced amnesia in monkeys: Effects of concurrent administration of physostigmine and methylphenidate with scopolamine. *Pharmacol. Biochem. Behav.*, 9:833-836, 1978.

Bartus, R.T. Physostigmine and recent memory effects in young and aged nonhuman primates. *Science*, 206:1087-1089, 1979.

Bartus, R.T. Cholinergic drug effects on memory and

cognition in animals. In: Poon, L.W. (ed.), *Aging in the 1980's: Psychological Issues.* Washington, DC: American Psychological Association, 1980, pp. 163-180.

Bartus, R.T., Dean, R.L., and Beer, B. Memory deficits in aged cebus monkeys and facilitation with central cholinomimetics. *Neurobiol. Aging,* 1:145-152, 1980.

Bartus, R.T., Dean, R.L., Beer, B., and Lippa, A.S. The cholinergic hypothesis of geriatric memory dysfunction. *Science,* 217:408-417, 1982.

Bartus, R.T., Dean, R.L., Sherman, K.A., Friedman, E., and Beer, B. Profound effects of combining choline and piracetam on memory enhancement and cholinergic function in aged rats. *Neurobiol. Aging,* 2:105-111, 1981.

Bartus, R.T., and Johnson, H.R. Short-term memory in the rhesus monkey: Disruption from the anticholinergic scopolamine. *Pharmacol. Biochem. Behav.,* 5:39-46, 1976.

Bierkamper, G.G., and Goldberg, A.M. The effect of choline on the release of acetylcholine from the neuromuscular junction. In: Barbeau, A., Growden, J.H., Wurtman, R.J. (eds.), *Nutrition and the Brain, Vol. 5.* New York:Raven Press, 1979, pp. 243-251.

Bird, E.D., and Iverson, L.L. Huntington's Chorea: Postmortem measurement of glutamic acid decarboxylase, choline acetyltransferase, and dopamine in basal ganglia. *Brain,* 97:457-474, 1974.

Blessed, G., Tomlinson, B.E., and Roth, M. The association between quantitative measures of dementia and of senile change in the cerebral gray matter of elderly subjects. *Br. J. Psychiatry,* 114:797-811, 1968.

Bowen, D.M., and Davison, A.N. Biochemical changes in the cholinergic system of the aging brain and in senile dementia. *Psychol. Med.,* 10:315-318, 1974.

Boyd, W.D., Graham-White, J., Blackwood, G., Glen, I., and McQueen, J. Clinical effects of choline in Alzheimer's senile dementia. *Lancet,* 2:711, 1977.

Brinkman, S.D., Pomara, N., Goodnick, P.J., Barnett, N., and Domino, E.F. A dose-ranging study of lecithin in the treatment of primary degenerative dementia (Alzheimer's disease). *J. Clin. Psychopharmacol.,* 2:281-285, 1982b.

Brinkman, S.D., Smith, R.C., Meyer, J.S., Vroulis, G., Shaw, T., Gordon, J.R., and Allen, R.H. Lecithin and memory training in suspected Alzheimer's disease. *J. Gerontol.,* 37:4-9, 1982a.

Brody, H. Organization of the cerebral cortex. *J. Comp. Neurol.,* 102:511-556, 1955.

Caine, E.D. Cholinomimetic treatment fails to improve memory disorders. *N. Engl. J. Med.,* 303:585-586, 1980.

Carlsson, A., Adolfsson, R., Aquilonius, S.M., Gottfries, C.G., Oreland, L., Svennerholm, L., and Winbald, B. In: Goldstein, M., Lieberman, A., Caine, D.B., and Thorner, M.O. (eds.), *Ergot Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects.* New York:Raven Press, 1980.

Christie, J.E., Blackburn, I.M., Glen, A., Zeisel, S., Shering, A., and Yates, C.M. Effects of choline and lecithin on CSF choline levels and in cognitive funcion in patients with presenile dementia of the Alzheimer type. In: Barbeau, A., Growden, H., and Wurtman, J. (eds.), *Nutrition and the Brain, Vol. 5.* New York:Raven Press, 1979, pp. 377-389.

Christie, J.E., Shering, A., Ferguson, J., and Glen, A.I.M. Physostigmine and arecoline: Effects of intravenous infusions in Alzheimer presenile dementia. *Br. J. Psychiatry,* 138:46-50, 1981.

Cohen, E.L., and Wurtman, R.J. Brain acetylcholine: Increase after systemic choline administration. *Life Sci.,* 16:1095-1099, 1975.

Corsellis, J.A.N. Aging and the dementias. In: Blackwood, W., and Corsellis, J.A.N. (ed.), *Greenfield's Neuropathology.* London:Edward Arnold, 1976, pp. 796-848.

Davies, P. Studies on the neurochemistry of central cholinergic systems in Alzheimer's disease. In: Katzman, R., and Terry, R. (eds.), *Alzheimer's Disease, Senile Dementia, and Related Disorders* (Series: *Aging, Vol. 7*). New York:Raven Press, 1978, pp. 453-459.

Davies, P. Biochemical changes in Alzheimer's disease senile dementia: Neurotransmitters in senile dementia of the Alzheimer's type. In: Katzman, R. (ed.), *Congenital and Acquired Cognitive Disorders.* New York:Raven Press, 1979a, pp. 153-160.

Davies, P. Neurotransmitter-related enzymes in senile dementia of the Alzheimer's type. *Brain Res.,* 171:319-326, 1979b.

Davies, P., and Terry, R.D. Cortical somatostatin-like immunoreactivity in cases of Alzheimer's disease and senile dementia of the Alzheimer's type. *Neurobiol Aging,* 2:9-14, 1981.

Davies, P., and Verth, A.H. Regional distribution of muscarinic acetylcholine receptor in normal and Alzheimer's type dementia brains. *Brain Res.,* 138:385-392, 1978.

Davis, B.M., Mohs, R.C., Levy, M.I., Greenwald, B.S., Rosen, W.G., Mathé, A.A., Deutsch, S.I., Horvath, T.B., and Davis, K.L. Neuroendocrine and CSF measures in Alzheimer's disease. Presented at the American College of Neuropsychopharmacology Annual Meeting, December 15-17, 1982.

Davis, K.L. Cholinergic deficit-based therapy in Alzheimer's disease. Presented at Cold Spring Harbor Symposium on Alzheimer's Disease, October 24-25, 1982.

Davis, K.L., Berger, P.A., Hollister, L.E., and Barchas, J.D. Cholinergic involvement in mental disorders (minireview). *Life Sci.,* 22(24):2171-2178, 1978.

Davis, K.L., Hollister, L.E., Overall, J., Johnson, A., and Train, K. Physostigmine: Effects on cognition and affect in normal subjects. *Psychopharmacology (Berlin),* 51:23-27, 1976.

Davis, K.L., Hsieh, J.Y-K, Levy, M.I., Horvath, T.B., Davis, B.M., and Mohs, R.C. Cerebrospinal fluid acetylcholine, choline, and senile dementia of the Alzheimer type. *Psychopharmacol. Bull.*, 18:193-195, 1982.

Davis, K.L., and Mohs, R.C. Enhancement of memory processes in Alzheimer's disease with multiple dose intravenous physostigmine. *Am. J. Psychiatry*, 139:1421-1424, 1982.

Davis, K.L., Mohs, R.C., Davis, B.M., Horvath, T.B., and Levy, M.I. Oral physostigmine in Alzheimer's disease. Presented at the American College of Neuropsychopharmacology, Annual Meeting, Dec. 15-17, 1982.

Davis, K.L., Mohs, R.C., and Tinklenberg, J.R. Enhancement of memory by physostigmine. *N. Engl. J. Med.*, 301:946, 1979.

Davis, K.L., Mohs, R.C., Tinklenberg, J.R., Pfefferbaum, A., Hollister, L.E., and Kopell, B.S. Physostigmine: Improvement of long-term memory processes in normal subjects. *Science*, 201:272-274, 1978.

de Kaban, A.S., and Sadowsky, D. Changes in brain weights during the span of human life: Relation of brain weights to body heights and body weights. *Ann. Neurol.*, 4:345-356, 1978.

Drachman, D.A. Memory and cognitive function in man: Does the cholinergic system have a specific role? *Neurology*, 27:783-790, 1977.

Drachman, D.A., Glosser, G., Fleming, P., and Longenecker, G. Memory decline in the aged: Treatment with lecithin and physostigmine. *Neurology*, 32:944-950, 1982.

Drachman, D.A., and Leavitt, J. Human memory and the cholinergic system. *Arch. Neurol.*, 30:113-121, 1974.

Drachman, D.A., and Sahakian, B.J. Memory and cognitive function in the elderly: A preliminary trial of physostigmine. *Arch. Neurol.*, 37:674-675, 1980.

Etienne, P., Dastoor, D., Gauthier, S., Ludwick, R., and Collier, B. Alzheimer's disease: Lack of effect of lecithin treatment for three months. *Neurology*, 31:1552-1554, 1981.

Etienne, P., Gauthier, S., Dastoor, D., Collier, B., and Ratner, J. Lecithin in Alzheimer's disease. *Lancet*, 2:1206, 1978a.

Etienne, P., Gauthier, S., Johnson, G., Collier, B., Mendis, T., Dastoor, D., and Cole, M. Clinical effects of choline in Alzheimer's disease. *Lancet*, 1:508-509, 1978.

Ferris, S.H., Sathanathan, G., Reisberg, B., and Gershon, S. Long-term choline treatment of memory-impaired elderly patients. *Science*, 205:1039-1040, 1979.

Growdall, P., Dyksen, M.W., Lazarus, L.W., Davis, J.M., Kahn, R.L., Jope, R., Finkel, S., and Rattan, P. Choline bitartrate treatment of Alzheimer-type dementias. *Commun. Psychopharmacol.*, 4:141-145, 1980.

Lund, G. Cholinergic receptor loss in brains of aging mice. *Life Sci.*, 26:371-375, 1980.

Odman, E., Sherman, K.A., Ferris, S.H., Reisberg, B., Cartus, R.T., and Schneck, M.K. Clinical response to choline plus piracetam in senile dementia: Relation to red-cell choline levels. *N. Engl. J. Med.*, 304:1490-1491, 1981.

Fuld, P.A., Katzman, R., Davies, P., and Terry, R.D. Intrusions as a sign of Alzheimer dementia: Chemical and pathological verification. *Ann. Neurol.*, 11:155-159, 1982.

Gibson, G.E., Peterson, C., and Jenden, D.J. Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitrous oxide. *Science*, 213:674-676, 1981.

Gibson, G.E., Peterson, C., and Sansone, J. Neurotransmitter and carbohydrate metabolism during aging and mild hypoxia. *Neurobiol. Aging*, 2:165-172, 1981.

Goldberg, A.M. The interaction of neuronal activity and choline transport on the regulation of acetylcholine synthesis. In: Corkin, S., Davis, K.L., Growden, J.H., Usdin, E., and Wurtman, R.J. (eds.), *Alzheimer's Disease: A Report of Progress in Research* (Series: *Aging, Vol. 19*). New York:Raven Press, 1982, pp. 327-330.

Gottfries, C.G. Biochemical aspects of dementia. In: Van Praag, H., Lader, M.H., Rafaelsen, O.J., and Sachar, E.J. (eds.), *Handbook of Biologic Psychiatry, Vol. IV: Brain Mechanisms and Abnormal Behavior*. New York:Marcel Dekker, Inc., 1980.

Haga, T., and Noda, H. Choline uptake systems of rat brain synaptosomes. *Biochem. Biophys. Acta*, 291:564-575, 1973.

Haubrich, D.R., Wang, P.F.L., and Wedeking, P. Role of choline in biosynthesis of acetylcholine. *Fed. Proc.*, 33:477, 1974.

Heston, L.L., and Mastri, A.R. Age at onset of Pick's and Alzheimer's dementia: Implications for diagnosis and research. *J. Gerontology*, 37:422-424, 1982.

Hökfelt, T., Johansson, O., Ljungdahl, A., Lundberg, J.M., and Schultzberg, M. Peptidergic neurones. *Nature*, 284:515-522, 1980.

James, T.C., and Kanungo, M.S. Alterations in atropine sites of the brain of rats as a function of age. *Biochem. Biophys. Res. Commun.*, 72:170-175, 1976.

Jenden, D.J., Roch, M., and Booth, R.A. Simultaneous measurement of endogenous and deuterium-labelled trace variants of choline and acetylcholine in sub-picomolar quantities by gas chromatography/mass spectrometry. *Anal. Biochem.*, 55:438, 1973.

Jervis, G.A. Alzheimer's disease. In: Minckler, J. (ed.), *Pathology of the Nervous System, Vol. 2*. New York:McGraw Hill, 1971, p. 1385.

Katzman, R. The prevalence and malignancy of Alzheimer disease. *Arch. Neurol.*, 33:217-218, 1976.

Kay, D.W.K. The epidemiology and identification of brain deficit in the elderly. In: Eisdorfer, C., and Friedel, R.O. (eds.), *Cognitive and Emotional Disturbance in the Elderly*. Chicago:Year Book Medical Publishers, 1977, pp. 11-26.

Kaye, W.H., Sitaram, N., Weingartner, H., Ebert, M.H., Smallberg, S., and Gillan, J. Modest facilitation of

memory in dementia with combined lecithin and anticholinesterase treatment. *Biol. Psychiatry*, 17:275-280, 1982.

Kral, V.A. Benign senescent forgetfulness. In: Katzman, R., Terry, R.D., and Bick, K.L. (eds.), *Alzheimer's Disease: Senile Dementia and Related Disorders* (Series: *Aging, Vol. 7*). New York:Raven Press, 1978, pp. 47-51.

Lippa, A.S., Critchett, D.J., Ehlert, F., Yamamura, H.I., Enna, S.J., and Bartus, R.T. Age-related alterations in neurotransmitter receptors: An electrophysiological and biochemical analysis. *Neurobiol. Aging*, 2:1-6, 1981.

Lippa, A.S., Pelham, R.W., Beer, B., Critchett, D.J., Dean, R.L., and Bartus, R.T. Brain cholinergic dysfunction and memory in aged rats. *Neurobiol. Aging*, 1:13-19, 1980.

Lundberg, J.M., Hedlund, B., and Bartfai, T. Vasoactive intestinal polypeptide enhances muscarinic ligand binding in cat submandibular salivary gland. *Nature*, 295:147-149, 1982.

Lundh, H., and Thesleff, S. The mode of action of 4-aminopyridine and guanidine on transmitter release from motor nerve terminals. *Eur. J. Pharmacol.*, 42:411-412, 1977.

McGeer, E.G., and McGeer, P.L. In: Ordy, J.M., and Brizzee, K.R. (eds.), *Advances in Behavioral Biology Vol. 16, Neurobiology of Aging*. New York:Plenum, 1975, p. 287.

McGeer, P.L., and McGeer, E.G. Enzymes associated with the metabolism of catecholamines, acetylcholine and GABA in human controls and patients with Parkinson's disease and Huntington's chorea. *J. Neurochem.*, 26:65-76, 1976.

Mesulam, M., Mufson, E.J., Levey, A.I., and Wainer, B.H. Alzheimer's disease and the cholinergic innervation of neocortex by the nucleus basalis of Meynert (Ch. 4). Presented at Cold Spring Harbor Symposium on Alzheimer's Disease, October 24-27, 1982.

Millington, W., and Goldberg, A.M. Precursor dependence of acetylcholine release from rat cortex *in vitro*. *Fed. Proc.*, 40:268, 1981.

Mohs, R.C., and Davis, K.L. A signal detectability analysis of the effect of physostigmine on memory in patients with Alzheimer's disease. *Neurobiol. Aging*, 3:105-109, 1982.

Mohs, R.C., Davis, K.L., Tinklenberg, J.R., and Hollister, L.E. Choline chloride effects on memory in the elderly. *Neurobiol. Aging*, 1:21-25, 1980.

Mohs, R.C., Davis, K.L., Tinklenberg, J.R., Hollister, L.E., Yesavage, J.A., and Kopell, B.S. Choline chloride treatment of memory deficits in the elderly. *Am. J. Psychiatry*, 136:1275-1277, 1979.

Mohs, R.C., Rosen, W.G., and Davis, K.L. Defining treatment efficacy in patients with Alzheimer's disease. In: Corkin, S., Davis, K.L., Growdon, J.H., Usdin, E., and Wurtman, R.J. (eds.), *Alzheimer's Disease: A Report of Progress in Research*. (Series: *Aging, Vol. 19*.). New York:Raven Press, 1982, pp. 351-356.

Morin, A.M., and Wasterlaine, C.G. Aging and rat brain muscarinic receptors as measured by quinuclidinyl benzilate binding. *Neurochem. Res.*, 5:301-308, 1980.

Muramoto, O., Sugishita, M., Sugita, H., and Toyokura, Y. Effect of physostigmine on constructional and memory tasks in Alzheimer's disease. *Arch. Neurol.*, 36:501-503, 1979.

Nickolson, V.J., and Wolthuis, O.L. Effect of the acquisition-enhancing drug piracetam on rat cerebral energy metabolism. Comparison with Nafidrofuryl and methamphetamine. *Biochem. Pharmacol.*, 25:2241-2244, 1976.

Nordberg, A., Adolfsson, R., Aquilonius, S.M., Marklund, S., Oreland, L., and Winbald, B. Brain enzymes and acetylcholine receptors in dementia of Alzheimer type and chronic alcohol abuse. In: Amaducci, L., Davison, A.N., and Antuono, P. (eds.), *Aging of the Brain and Dementia* (Series: *Aging, Vol. 13*). New York:Raven Press, 1980, pp. 169-171.

Perry, E.K. The cholinergic system in old age and Alzheimer's disease. *Age Ageing*, 9:1-8, 1980.

Perry, E.K., Gibson, P.H., Blessed, G., Perry, R.H., and Tomlinson, B.E. Neurotransmitter enzyme abnormalities in senile dementia. *J. Neurol. Sci.*, 34:247-265, 1977.

Perry, E.K., Perry, R.H., Blessed, G., and Tomlinson, B.E. Necropsy evidence of central cholinergic deficits in senile dementia. *Lancet*, 1:189-191, 1977.

Perry, E.K., Perry, R.H., Gibson, P.H., Blessed, G., and Tomlinson, B.E. A cholinergic connection between normal aging and senile dementia in human hippocampus. *Neurosci. Lett.*, 6:85-89, 1977.

Perry, E.K., Tomlinson, B.E., Blessed, G., Bergman, K., Gibson, P.H., and Perry, R.H. Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J.*, 2:1457-1459, 1978.

Peters, B.H., and Levin, H.S. Effects of physostigmine and lecithin on memory in Alzheimer disease. *Ann. Neurol.*, 6:219-221, 1979.

Plum, F. Dementia, an approaching epidemic. *Nature*, 279:372-373, 1979.

Price, D.L., Whitehouse, P.J., Struble, R.G., Clark, A.W., Coyle, J.T., DeLong, M.R., and Hedreen, J.C. Basal forebrain cholinergic systems in Alzheimer's disease and related dementias. *Neurosci. Commentaries*, 1:84-85, 1982.

Reisine, T.D., Yamamura, H.I., Bird, E.D., Spokes, E., and Enna, S.J. Pre- and post-synaptic neurochemical alterations in Alzheimer's disease. *Brain Res.*, 149:447-481, 1978.

Renvoize, E.B., and Jerram, T. Choline in Alzheimer's disease. *N. Engl. J. Med.*, 301:330, 1979.

Ron, M.A., Toone, B.K., Garralda, M.E., and Lishman,

W.A. Diagnostic accuracy in presenile dementia. *Br. J. Psychiatry*, 134:161-168, 1979.

Rossor, M.N., Emson, P.C., Mountjoy, C.Q., Roth, M., and Iversen, L.L. Reduced amounts of immunoreactive somatostatin in the temporal cortex in senile dementia of Alzheimer type. *Neurosci. Lett.*, 20:373-377, 1980.

Rossor, M.N., Fahrenkrug, J., Emson, P.C., Mountjoy, C., Iversen, L., and Roth, M. Reduced cortical CAT activity in SDAT is not accompanied by changes in VIP. *Brain Res.*, 201:249, 1980.

Roth, M., and Hopkins, B. Psychological test performance in patients over sixty. Senile psychosis and affective disorders of old age. *J. Ment. Sci.*, 99:439, 1953.

Sherman, K., Kuster, J.E., Dean, R.L., Bartus, R.T., and Friedman, E. Presynaptic cholinergic mechanisms in brain of aged rats with memory impairments. *Neurobiol. Aging*, 2:99-104, 1981.

Signoret, J., Whiteley, A.L., and L'hermitte, F. Influence of choline on amnesia in early Alzheimer's disease. *Lancet*, 2:837, 1978.

Sims, N.R., Mareck, K.L., Bowen, D.M., and Davison, A.N. *J. Neurochem.*, 38:488, 1982.

Sims, N.R., Smith, C.C.T., Davison, A.M., Bowen, D.M., Flack, R.H.A., Snowden, J.S., and Neary, D. Glucose metabolism and acetylcholine synthesis in relation to neuronal activity in Alzheimer's disease. *Lancet*, 1:333-336, 1980.

Sitaram, N., Weingartner, H., and Gillin, J.C. Human serial learning: Enhancement with arecholine and impairment with scopolamine correlated with performance on placebo. *Science*, 201:274-276, 1978.

Smith, C.M., and Swash, M. Physostigmine in Alzheimer's disease. *Lancet*, 1:42, 1979.

Smith, C., Swash, M., Exton-Smith, A., Phillips, M.J., Overastall, P.W., Piper, M.E., and Bailey, M.R. Choline therapy in Alzheimer's disease. *Lancet*, 2:318, 1978.

Spillane, J.A., White, P., Goodhardt, M.J., Flack, R.H.A., Bowen, D.M., and Davison, A.N. Selective vulnerability of neurons in organic dementia. *Nature*, 226:558-559, 1977.

Spokes, S. An analysis of factors influencing measurements of dopamine, noradrenaline, glutamate decarboxylase and choline acetylase in human postmortem brain tissue. *Brain*, 102:333-346, 1979.

Strong, R., Hicks, P., Hsu, L., Bartus, R.T., and Enna, S.J. Age-related alterations in the rodent brain cholinergic system and behavior. *Neurobiol. Aging*, 1:59-63, 1980.

Struble, R.G., Cork, L.C., Whitehouse, P.J., and Price, D.L. Cholinergic innervation in neuritic plaques. *Science*, 216:413-415, 1982.

Sullivan, E.V., Shedlack, K.J., Corkin, S., and Growdon, J.H. Physostigmine and lecithin in Alzheimer's disease. In: Corkin, S., Davis, K.L., Growdon, J.H., Usdin, E., and Wurtman, R.J. (eds.), *Alzheimer's Disease: A Report of Progress in Research* (Series: *Aging, Vol. 19*). New York:Raven Press, 1982, pp. 361-368.

Summers, W.K., Viesselman, J.O., Marsh, G.M., and Candelora, K. Use of THA in treatment of Alzheimer-like dementia: Pilot study in twelve patients. *Biol. Psychiatry*, 16:145-153, 1981.

Thal, L.J., Masur, D.M., Fuld, P.A., Sharpless, N.S., and Davies, P. Oral physostigmine and lecithin improve memory in Alzheimer's disease. *Psychopharmacol. Bull.* (In press)

Thal, L.J., Rosen, W., Sharpless, N.S., and Crystal, H. Choline chloride fails to improve cognition in Alzheimer's disease. *Neurobiol. Aging*, 2:205-208, 1981.

Torack, R.M. *The Pathological Physiology of Dementia*. Berlin:Springer-Verlag, 1978, pp. 17-25.

Vroulis, G., Smith, R.C., Brinkman, S., Schoolar, J., and Gordon, J. Effects of lecithin on memory and behavior in Alzheimer's type dementia. *Psychopharmacol. Bull.*, 17:127-128, 1981.

Wecker, L., Dettbarn, W.D., and Schmidt, D.E. Choline administration: Modification of the central actions of atropine. *Science*, 199:86, 1978.

White, P., Goodhardt, M.J., Kent, J.P., Hiley, C.R., Carrasco, L.H., Williams, J.E., and Bowen, D.M. Neocortical cholinergic neurons in elderly people. *Lancet*, 1:668-671, 1977.

Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T., and DeLong, M.R. Alzheimer's disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Ann. Neurol.*, 10:122, 1981.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T., and DeLong, M.R. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science*, 215:1237-1239, 1982.

Whiteley, A., Signoret, J.L., Agid, Y., and L'hermitte, F. *Rev. Neurol.*, 135:565, 1979.

Wilcock, G.K., and Esiri, M.M. Plaques, tangles, and dementia: A quantitative study. *J. Neurol. Sci.*, 56:343-356, 1982.

Wilcock, G.K., Esiri, M.M., Bowen, D.M., and Smith, C.C.T. The nucleus basalis in Alzheimer's disease: Cell counts and cortical biochemistry. *Neuropath. Appl. Neurobiol.* (In press)

Wisniewski, H.M., and Kozlowski, P.B. Evidence for blood-brain barrier changes in senile dementia of the Alzheimer type (SDAT). In: Sinek, F.M., and Merril, C.R. (eds.), *Alzheimer's Disease, Down's Syndrome, and Aging* (Ann. NY Acad. Sci. Vol. 396). New York, 1982, pp. 119-129.

Wurtman, R.J., Magil, G.S., and Reinstein, D.K. Piracetam diminished hippocampal acetylcholine levels in rats. *Life Sci.*, 28:1091-1093, 1981.

Yamamura, H.I., and Snyder, S. High-affinity transport of choline into synaptosomes of rat brain. *J. Neurochem.*, 21:1355-1374, 1973.

EXHIBIT 31

GALANTHAMINE:
Another Look at an Old Cholinesterase Inhibitor

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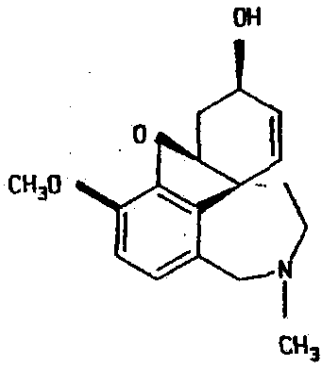
I. INTRODUCTION

At the present time the cholinergic deficiency hypothesis involving nucleus basalis and related areas in the basal forebrain of Alzheimer's patients provides the most practical means of developing new therapies. In view of the interest in experimental treatments using physostigmine and tetrahydroaminoacridine, it seems appropriate to search for other cholinesterase inhibitors (ChEI) that readily penetrate the blood-brain-barrier. Galanthamine is such an agent. The purpose of this brief review is to stimulate further examination of this agent.

II. CHEMICAL ISOLATION AND CHARACTERIZATION

The early chemical and pharmacognosy literature is a little confusing on the precise details of the chemical isolation and characterization of the alkaloid galanthamine. This reviewer has not had the opportunity to check all of the original literature in the native language of the author(s). What follows is an attempt to briefly summarize some of the papers that have been quoted in this field.

Galanthamine apparently was first isolated in the USSR from the caucasian snowdrop *Galanthus woronowii* by Proskurnina and Yakovlena (1947) and Proskurnina and Areshkina (1952, 1953). Proskurnina and Areshkina (1947, 1948) reported on the presence of galanthamine in *Galanthus nivalis* which also contained other alkaloids including lycorine, tazettine, etc. Proskurnina and Yakovlena (1952, 1953) reported on the further isolation of galanthamine from *Galanthus woronowii*. In these early years there was some confusion over the precise chemical structures of the alkaloids isolated. For example, Uyeo and Kobayashi (1953) identified galanthamine as identical with lycoramine extracted from *Lycoris radita*. The identity of these compounds was also emphasized by Boit and Ehmke (1955, 1956) and Briggs *et al.* (1956). Kobayashi *et al.* (1956) suggested that the original assigned structures of galanthamine and lycoramine differed in that the structure first assigned to galanthamine was inconsistent with their experimental results. Galanthamine was again extracted from *Galanthus nivalis var. gracilis* in Bulgaria by Bubeva-Ivanova (1957). Bossier *et al.* (1960) reviewed the botanical and chemical data then available. They discussed at length the different botanical sources of galanthamine and related alkaloids as well as their chemical structures. In 1962 Barton and Kirby reported the chemical synthesis of galanthamine. In 1964 Williams and Rogers described the structure of galanthamine methiodide. Kametani *et al.* (1971) reported on a new alternative synthesis of galanthamine. The chemical structure of galanthamine as described in the Merck Index (1983) is shown on the following page.



Galanthamine

Because of its heterocyclic structure as a phenantridine derivative and its crude relationship in chemical structure to codeine, the structure of galanthamine has been compared to that of codeine. However, their pharmacological properties are very different. Galanthamine is also known by several other generic names including galantamine(sic) and lycoremine(sic). According to the Merck Index (1983), the chemical composition of galanthamine is 4a,5,9,10,11,12- hexahydro- 3-methoxy- 11-methyl- 6H-benzofuro [3a,3,2-ef][2] benzazepin-6-ol. As can be seen from its chemical structure, galanthamine is a tertiary amine that would be expected to penetrate readily the blood-brain-barrier.

III. SOURCES

Currently galanthamine hydrobromide is available under the trade name Nivalin, from Pharmachim State Economic Association, 16 Iliensko, Chaussée, Sofia, Bulgaria. The clinical indications for the use of Nivalin as specified by the company are obviously overly inclusive, including neuritis, especially facial, radiculitis, radiculoneuritis, polyneuritis, poliomyelitis, myopathies including myasthenia gravis pseudoparalytica, progressive muscular dystrophy, spinal and neuritic muscular atrophy. Additional indications are spastic pareses and paralysis, and other sequelae of lesions of the central nervous system of vascular, inflammatory, toxic, and traumatic origin including cerebral apoplexy, meningitis, meningoencephalitis and myelitis. As would be expected from a ChEI, Nivalin very early was shown to be effective in neuromuscular diseases, especially myasthenia gravis (Pestel, 1961). Gopel and Bertram (1971) also have reported on their favorable experiences using Nivalin in some neurologic diseases. In addition, Nivalin has been indicated for glaucoma and as an anticurare agent in anesthesia as well as in diseases with decreased tone of smooth musculature of the urinary bladder and gastrointestinal tract. In view of the cholinergic deficit hypothesis in Alzheimer's disease, one would expect that galanthamine was tried in this condition although it is not well described in the literature available to this reviewer.

The contraindications for Nivalin are said to include epilepsy, bronchial asthma, cardiac diseases with bradycardia, and hyperkinesia. Interestingly, side effects are said to occur very rarely and include increased salivation, nausea, dizziness and bradycardia (Cozanitis *et al.*, 1973). Although a number of pharmaceutical companies in western Europe have been interested in Nivalin, to date, to our knowledge, none of these companies are currently supplying galanthamine or its salts.

In the USSR galanthamine is recommended to be administered s.c. as the hydrobromide in doses of 10 and 20 mg maximally. The agent is used primarily as an antimyasthenic and antagonist of curare-like effects as well as for a variety of central nervous system disorders.

IV. PHARMACOLOGY

A Cholinesterase Inhibition

Irwin and Smith (1960a,b) and Boissier and Lesbros (1962) compared the degree of acetylcholinesterase inhibition (AChEI) in skeletal muscle and brain, and butyrylcholinesterase inhibition (BChEI) in plasma. They were able to show that muscle acetylcholinesterase (AChE) activity was inhibited by galanthamine to a greater extent than that produced by pyridostigmine, although clearly less than that of neostigmine. These investigators concluded that galanthamine and related alkaloids are of interest because of their potential therapeutic usefulness as ChEI. Galanthamine was more effective in inhibiting plasma BChE than it was muscle AChE. Pyridostigmine was much less potent in inhibiting AChE.

B Antagonism of Central Anticholinergic Effects

Plaitakis and Duvoisin (1983) collected evidence from the literature that Homer's Moly is *Galanthus nivalis* of which the active ingredient is galanthamine. They suggested that Circe's poisonous plants included stramonium which was thought to be used by Circe to induce amnesia in Odysseus' crew. Plaitakis and Duvoisin suggest that the description of Moly as an antidote in Homer's Odyssey may represent the oldest recorded use of a ChEI to reverse the central anticholinergic syndrome.

Baraka and Harik (1977) determined the effectiveness of 0.5 mg/kg of galanthamine hydrobromide given i.v. in reversing the central anticholinergic syndrome induced by 2 mg of scopolamine i.v. in 10 adult volunteers. Following the administration of i.v. scopolamine, the volunteers became drowsy within 10 min, reaching a peak effect within 30-40 min. The drowsiness was sometimes associated with disorientation, visual hallucinations, and delirium. The administration of 0.5 mg/kg of galanthamine hydrobromide i.v. reversed the central effects of scopolamine. The subjects became alert within 5-10 min and were completely awake after 30 min. As would be expected following the administration of scopolamine, the pulse rate increased from a control of 60-80 to ~110-130/min. After galanthamine was given, the heart rate returned to control levels of 60-70/min. Two hr later, the subjects felt more alert than usual and did not feel sleepy. These investigators also showed that the EEG changes associated with scopolamine (which included a decrease in *alpha* rhythm) reached their maximum within 30 min and, in addition, the *alpha* rhythm was replaced by disorganized 4-6 Hz *theta* activity of moderate to low amplitude. Again, galanthamine promptly reversed the EEG effects of scopolamine. Although the authors concluded that galanthamine produced a long lasting reversal of the central anticholinergic syndrome, from their study one could only determine that the effect of the drug lasted 2 hr with no evidence that it had a much longer duration of action.

Cozanitis (1977) also reported on the effectiveness of galanthamine hydrobromide in treating the central anticholinergic syndrome induced by scopolamine. A 31 year old man who ingested an unknown quantity of scopolamine time release tablets exhibited a marked anticholinergic syndrome. He was flushed, semicomatose and only slightly reactive to pain. In addition, his respiration was slow and shallow. His breath smelled of ethyl alcohol. After gastric lavage a large mass of tablets was recovered, but the patient became delirious requiring restraints. Galanthamine hydrobromide, in a dose of 20 mg i.v., within 10 min

produced a dramatic improvement. The patient was observed over the next 10 hr and was free of psychiatric and physiological manifestations of poisoning. His breathing rate rose from 14 to 20/min. Cozanitis emphasized that galanthamine hydrobromide appeared to be a long acting ChEI in antagonizing the central effects of scopolamine.

C. Antagonism of Skeletal Neuromuscular Blockade

The anticurare action of galanthamine was described by Mashkovsky (1955). Boissier *et al.* (1960) showed the potency of antagonism by galanthamine of nondepolarizing muscle relaxants is 1/10 that of neostigmine. Galanthamine seemed to act faster but was shorter acting. It prolonged the action of succinylcholine neuromuscular blockade. Bradycardia was seen in the frog, rabbit, and dog, and hypotension in the dog. As expected, galanthamine enhanced acetylcholine (ACh) provoked contraction of smooth muscle and increased the contraction of striated muscle after maximal direct and indirect stimulation.

When given s.c., the effect of galanthamine is somewhat slower and less potent than neostigmine but is said to be longer lasting and less toxic (Pestel, 1961). The quaternary derivative of galanthamine is more potent than its non-quaternary hydrobromide, being about four times as potent as a ChEI. Paskov *et al.* (1964) showed in experimental animals that galanthamine was effective in antagonizing morphine induced depression. The LD_{100} of galanthamine in rats is 45 mg/kg, in rabbits 12 mg/kg, and in cats 60 mg/kg (Stoyanov, 1964a,b). Stoyanov and Vulchanova (1963) reported on the efficacy of galanthamine as a curare antagonist in a large number of patients in Bulgaria. In addition, Mayrhofer (1966) reported his favorable clinical experiences with galanthamine as a curare antagonist.

Wislicki (1967) provided additional evidence that galanthamine hydrobromide, which has advantages as an antagonist of nondepolarizing muscle relaxants should be studied in Western countries. He described the use of galanthamine in 24 patients. Galanthamine was about 1/10 as potent as neostigmine. Changes in pulse rate and blood pressure were slight and it was rarely necessary to inject atropine before galanthamine. Salivation had to be suppressed by atropine in only 1/7th of the cases studied.

Cozanitis and his colleague (Baraka and Cozanitis, 1973; Cozanitis, 1971, 1974) have provided extensive data that galanthamine is a very useful agent in reversing nondeplolarization block in man. Cozanitis *et al.* (1977) compared the potency of galanthamine and neostigmine in reversing d-tubocurarine blockade in the isolated rat diaphragm. Neostigmine appeared to be about 18 times as potent on a weight basis in the rat whereas the potency ratio *in vivo* in man is about 20 times greater than galanthamine. These investigators indicated that the potency differences appear to be determined on the basis of drug effects on neuromuscular transmission and not on pharmacokinetic differences.

D. General Pharmacology and Toxicity

Paskov summarized much of the general pharmacology of galanthamine in 1959. Boissier and his colleagues (1960) also described the pharmacological actions of galanthamine which indicate its effectiveness as a cholinergic agonist. Compared to neostigmine, galanthamine was much less potent but also appeared to be less toxic. In the mouse the toxicity of galanthamine hydrobromide compared to neostigmine was 16.5 times less after i.v. and 21.3 times less when given i.p. Subsequently, Boissier and Lesbros (1962) compared the AChEI actions of galanthamine with physostigmine, neostigmine, lycoramine methyl iodide, and

galanthamine methyl iodide. They were able to show that galanthamine itself was a ChEI of both serum BuChE as well as AChE from red blood cells and skeletal muscle. Galanthamine itself was relatively weak compared to neostigmine and physostigmine. The quaternary methyl iodide preparation was more potent relatively as a true AChEI compared to tertiary galanthamine hydrobromide. Kostowski and Gumulka (1968) suggested that galanthamine had ganglionic actions more like neostigmine than physostigmine and that more than muscarinc agonist actions were involved. However, in unanesthetized cats galanthamine caused marked EEG desynchronization in the neocortex and increased *theta* activity in the hippocampus which was completely abolished by atropine or benactyzine, suggesting a predominant muscarinic action in the brain. After mesencephalic lesions, the hippocampal *theta* rhythm previously induced by galanthamine was blocked (Mashkovsky and Illuchenok, 1961).

Cozanitis *et al.* (1983) studied the effects of galanthamine in a variety of animals and isolated organ preparations. They observed that galanthamine given s.c. had antinociceptive activity. This was compared to that induced by physostigmine and morphine. Naloxone blocked the antinociceptive effects of galanthamine but not that of physostigmine, suggesting that galanthamine had an opioid narcotic component not possessed by physostigmine. Both ChEIs produced analgesia in the mouse writhing test and potentiated the effect of morphine in the rat hotplate analgesic test. Although galanthamine produced analgesia in the intact animal, it failed to produce opioid-like activity in isolated organ preparations such as the longitudinal muscle strip of the guinea pig ileum, the mouse vas deferens, and the cat nictitating membrane. The overall data suggest that galanthamine may release endogenous opioids in the intact animal and that galanthamine-induced analgesic effects are partially antagonized by naloxone. These investigators also noted that galanthamine has a molecular configuration similar to codeine (Williams and Rogers, 1964) but galanthamine was not acting through a classic *mu* opioid mechanism but rather apparently as an unusual ChEI different from physostigmine. Cozanitis and Rosenberg (1974) compared the effects of galanthamine hydrobromide on dextromoramide-depressed respiration in rabbits. They studied this phenomenon in view of their previous experience (see below) of apnea reversal by galanthamine in a patient who ingested a large dose of dextromethoramide, methaqualone and diphenhydramine. Cozanitis and Rosenberg showed that a dose of 1 mg/kg or more of galanthamine was effective in antagonizing the respiratory depression. However, the action was slower in onset than that produced by 1 mg/kg of nalorphine.

Rupreht *et al.* (1983) studied the involvement of the central cholinergic and opioid systems in nitrous oxide withdrawal in mice. Mice were exposed to a mixture of 1.4 atm of nitrous oxide and 0.6 atm oxygen for a period of 60 min. After 60 min of exposure to this mixture, the pressure was reduced to the ambient level over a period of 1 min and each animal was tested for withdrawal convulsions. Physostigmine, galanthamine, and naloxone were compared in their ability to modify the withdrawal syndrome. Physostigmine, in a dose of 0.4 μg/g decreased to 17.5 min the period of susceptibility during which withdrawal convulsions were observed. A similar significant decrease was observed when the same dose of physostigmine was given 1 min after the nitrous oxide was discontinued. Over a 10 fold dose of galanthamine (5 μg/g) had similar properties and decreased to 33.3 min the period of time in which seizures could be elicited. The same dose of galanthamine, in contrast to physostigmine, failed to significantly affect the period of withdrawal convulsions when the galanthamine was given in the first min after withdrawal. This suggests that physostigmine has a more rapid onset of action than galanthamine. In addition, naloxone, 0.8 μg/g before nitrous oxide exposure significantly decreased the predisposition to seizures. However, the same dose of naloxone administered after the first min after nitrous oxide was discontinued failed to influence the duration in which the convulsant phenomenon could be elicited.

E. Antagonism of Drug-Induced Respiratory Depression

Paskov *et al.* (1964) found that galanthamine hydrobromide antagonized respiratory depression induced by morphine, meperidine, and dextromoramide irrespective of whether the animals' carotid bodies had been removed, suggesting that this was a direct central nervous system effect. These investigators also noted that intracisternal injections of galanthamine hydrobromide antagonized the respiratory depression induced by these narcotics. Stoyanov *et al.* (1965) and Stoyanov and Statkov (1968) reported galanthamine hydrobromide to antagonize steroid and fentanyl depression. Cozanitis and Toivakka (1974) used galanthamine hydrobromide to antagonize the respiratory depression of a 44 year old man who ingested a large quantity of methaqualone, diphenhydramine and dextromoramide. Respiration ceased almost immediately after admission and an endotracheal tube was passed to facilitate artificial ventilation. The i.v. administration of galanthamine hydrobromide, in a dose of 20 mg, within 20 min caused respiratory stimulation. An additional dose of 10 mg was even more effective. Cozanitis and Toivakka (1971) did a comparative clinical pharmacologic study of the effects of galanthamine hydrobromide and atropine-neostigmine in conscious volunteers. They reported that the electroencephalographic pattern after i.v. galanthamine bromide fulfills the requirement of an analeptic.

Tassonyi *et al.* (1976) showed that galanthamine hydrobromide in doses of 20-70 mg rapidly reversed postoperative apnea after neurolept analgesia. The patients regained consciousness, followed instructions, and moved well. However, 15-40 min after galanthamine hydrobromide administration suddenly the patients lost consciousness and breathing stopped. After about 20-60 min of artificial ventilation the patients gradually regained consciousness and their breathing became normal. Tassonyi *et al.* (1976) discussed the various possibilities for this phenomenon. Perhaps the most likely explanation is that galanthamine hydrobromide has a much shorter duration of action in man than has previously been recognized.

F. Endocrine effects

Cozanitis *et al.* (1980) compared the effects of galanthamine and neostigmine on plasma ACTH in patients undergoing surgical anesthesia. The patients required skeletal muscle relaxation in which galanthamine and neostigmine were indicated to reverse neuromuscular blockade. Following the administration of these agents to reverse nondepolarizing neuromuscular blockade, galanthamine, in contrast to neostigmine, statistically significantly elevated plasma ACTH, suggesting that the rise in plasma cortisol they also observed is ACTH dependent. They concluded that a peripheral cholinergic mechanism was not involved because galanthamine is a tertiary amine whereas neostigmine is a quaternary amine and therefore the latter would not be expected to penetrate the blood-brain-barrier.

G. Pharmacokinetics

Mihailova and Yamboliev (1986) described the pharmacokinetics of galanthamine hydrobromide (Nivalin) following single i.v. and oral doses to rats. The plasma samples were collected and the concentrations of the drug determined spectrophotofluorometrically. A two compartment open model was found to best describe the experimental data. Galanthamine had an elimination half life of about 40-50 min, a volume of distribution of over 2 l/kg, a plasma clearance of about 2 l/hr/kg and an oral availability of about 65%. Westra *et al.* (1986) studied the pharmacokinetics of galanthamine in 8 anesthetized patients. They showed that after an i.v. injection of 0.3 mg/kg the decrease in the serum concentration of galanthamine followed a biexponential curve. Serum

concentrations decreased very rapidly between 2 and 30 min with a $t_{\frac{1}{2}}$ of the *alpha* phase of 6.42 and then declined more slowly with a $t_{\frac{1}{2}}$ of the *beta* phase of 264 min. Total serum clearance of galanthamine amounted to 5.37 ml/min/kg and the renal clearance was 1.36 ml/min/kg. Cumulative urinary excretion of galanthamine between 0 and 48 hr after injection amounted to 28.0% of the administered dose. Biliary excretion of galanthamine during 24 hr amounted to 0.2% of the dose. There was no evidence of glucuronide or sulfate conjugation of galanthamine. These authors pointed out that the elimination half life of galanthamine was 264 min whereas the ChEIs neostigmine, pyridostigmine and endrophonium, currently used as antagonists of nondepolarizing neuromuscular blockade, have elimination half lives in man of respectively 80, 46, and 114 min. Paskov *et al.* reported that serum BuChE is inhibited for about 3 hr after the administration of galanthamine. In contrast, the inhibition of BuChE activity by neostigmine and pyridostigmine have been reported to be shorter.

Westra *et al.* (1986) concluded that from a pharmacokinetic point of view, galanthamine is suitable to reverse the unwanted and prolonged side effects of skeletal neuromuscular blocking agents but obviously further pharmacological studies are needed. Especially clear, however, from their pharmacokinetic analysis is the fact that galanthamine is not a very long acting compound. This finding is consistent with our own unpublished studies on the comparative effects of physostigmine, tetrahydroaminoacridine, and galanthamine in suppressing self-stimulation behavior in the rat where galanthamine is only as long acting as physostigmine but much less potent.

V. SUMMARY

Galanthamine is a tertiary cholinesterase inhibitor that has central nervous system actions. It is well known and widely used in eastern European countries. Although less potent than physostigmine and with a similar duration of action, it is claimed to be less toxic. Galanthamine deserves further study as a possible indirect cholinergic agonist treatment of the cognitive deficits in Alzheimer's disease.

VI. REFERENCES

Baraka, A. and Cozanitis, D. 1973. Galanthamine versus neostigmine for reversal of nondepolarizing neuromuscular block in man. Anesth. Analg. Cur. Res. 52: 832-836.

Baraka, A. and S. Harik. 1977. Reversal of central anticholinergic syndrome by galanthamine. J. Am. Med. Assoc. 238: 2293-2294.

Barton, D.H.R. and G.W. Kirby. 1962. Phenol oxidation and biosnthesis. Part V. The synthesis of galanthamine. Proc. Chem. Soc.: 806-817.

Boissier, J.-R., G. Combes, and J. Pagny. 1960. La galanthamine, puissant cholinergique naturel. I. Sources. Structure chimique. Caracterisation. Extraction. Toxicite. Action sur les fibres lisses. Extrait des Anneles pharmaceutiques francaises. 18: 888-900.

Boissier, J.-R. and J. Lesbros. 1962. La galanthamine, puissant cholinergique naturel. II. Activite anticholinesterasique de la galanthamine et de quelques derives. Extrait des Annales pharmaceutiques francaises 20: 150-155.

Boit, H.G. and H. Ehmke. 1955. Chem. Ber. 88: 1590.

Boit, H.G. and H. Ehmke. 1956. Chem. Ber. 89: 163 and Chem. Ber. 90: 725, 2197.

Briggs, C.K., P.F. Highet, R.S. Highet, and W.C. Wildman. 1956. J. Amer. Chem. Soc. 78: 2899.

Bubeva-Ivanova, L. 1957. Fidochemical Investigation of Galanthus nivalis var. gracilis. Farmatsiya (Sofia) 2: 23.

Cozanitis, D.A. 1971. Experiences with galanthamine hydrobromide as curare antagonist. Der Anaesthetist 20: 226-229.

Cozanitis, D.A. 1974. Galanthamine hydrobromide versus neostigmine. A plasma cortisol study in man. Anaesthesia 29: 163-168.

Cozanitis, D.A. 1977. Galanthamine hydrobromide, a longer acting anticholinesterase drug, in the treatment of the central effects of scopolamine (hyoscine). Anaesthesist 26: 649-650.

Cozanitis, D., A. Dessypris, and K. Nuuttila. 1980. The effect of galanthamine hydrobromide on plasma ACTH in patients undergoing anaesthesia and surgery. Acta anaesth. scand. 24: 166-168.

Cozanitis, D.A., T. Friedmann, and S. Fürst. 1983. Study of the analgesic effects of galanthamine, a cholinesterse inhibitor. Arch. Int. Pharmacodynamie Therap. 266: 229-238.

Cozanitis, D.A., K. Nuuttila, P. Karhunen, and A. Baraka. 1973. Changes in cardiac rhythm with galanthamine hydrobromide. Anaesthesist 22: 457-459.

Cozanitis, D.A. and P. Rosenberg. 1974. Preliminary experiments with galanthamine hydrobromide on depressed respiration. Anaesthesist 23 320-305.

Cozanitis, D.A., A.H.J. Schaf, and J. Van Den Akker. 1977. The potency ratio of galanthamine and neostigmine on the reversal of the d-tubocurarine block in the isolated rat diaphragm. Acta Anaesthesiologica Belgica 28: 53-60.

Cozanitis, D.A. and E. Toivakka. 1971. A comparative study of galanthamine hydrobromide and atropine/neostigmine in conscious volunteers. Der Anaesthesist 20: 416-421.

Cozanitis, D.A. and E. Toivakka. 1974. Treatment of resiratory depression with the anticholinesterase drug galanthamine hydrobromide. Anaesthesia 29: 581-584.

Gopel, W. and W. Bertram. 1971. Erfahrungen mit Nivalin in der neurologischen Therapie. Psychiat. Neurol. med Psychol. Leipzig 23: 712-718.

Irwin, R.L. and H.J. Smith III. 1960a. Cholinesterase inhibition by galanthamine and lycoramine. Biochem. Pharmacol. 3: 147-148.

Irwin, R.L. and H.J. Smith III. 1960b. The activity of galanthamine and related compounds. Arch. int. Pharmacodyn. 127: 314.

Kametani, T., C. Seino, K. Yamaki, S. Shibuya, K. Fukumoto, K. Kigasawa, F. Satoh, M. Hiiragi, and T. Hayasaka. 1971. Studies on the syntheses of heterocyclic compounds. Part CCCLXXVI. Alternative total syntheses of galanthamine and N-benzylgalanthamine iodide. J. Chem. Soc. 1043-1047.

Kobayashi, S., T. Shingu, and S. Uyeo. 1956. Structure of galanthamine and lycoramine. Chem. and Ind. (London) 177-178.

Kostowski, W. and W. Gumulka. 1968. Note on the ganglionic and central actions of galanthamine. Int. J. Neuropharmacol. 7: 7-14.

Mashkovsky, M.D. 1955. Effects of galanthamine on the acetylcholine sensitivity of skeletal musculature. Pharmacol. and Toxic. 18: 21.

Mashkovsky, M.D. and R.J. Illuchenok. 1961. On the problem of action of galanthamine on the central nervous system. Z. Nevropatol. i. Psichiatrii 61: 166-175.

Mayrhofer, O. 1966. Clinical experiences with diallyl-nor-toxiferine and the curare antidote galanthamine. Southern Med. J. 59: 1364-1368.

Merck Index, 10th Edition. 1983. Windholz, M., S. Budavari, R.F. Blumetti, and E.S. Otterbein, eds., No. 4210, galanthamine, pg. 620. Merck and Company, Rahway, NJ.

Mihailova, D. and Yamboliev, I. 1986. Pharmacokinetics of galanthamine hydrobromide (Nivalin^{R}) following single intravenous and oral administration in rats. Pharmacology 32: 301-306.

Paskov, D.S. 1959. Nivalin-Pharmakologicheska Karakteristika Sofia.

Paskov, D., H. Dobrev, and N. Nikoforonov. 1964. Antagonistic effect of Nivalin (galanthamine) versus morphine upon the respiratory center. Acta Inst.

Superioris Med. (Sofia) 42: 1.
Pestel, M. 1961. Une nouvelle médication de la myasthénie et des dystrophies neuromusculaires: la Nivaline. Presse méd. 69: 182.
Plaitakis, A. and R.C. Duvoisin. 1983. Homer's moly identified as Galanthus nivalis L: Physiologic antidote to stramonium poisoning. Clin. Neuropharmacol. 6: 1-5.
Proskurnina, N.F. and L.Y. Areshkina. 1947. J. Chim. Gen. USSR 17: 1216 and 1948. Chem. Abst. 42: 1595b.
Proskurnina, N.V. and L.Y. Areshkina. 1952. Alkaloids of Galanthus woronovi II. Isolation of a new alkaloid. Zhur. Obshchei Khim. 22: 1899; and 1953. Chem. Abst. 47: 6959c.
Proskurnina, N.F. and A.P. Yakovlena. 1947. On the alkaloid Galanthus woronowi. Zh. Obshch. Kalim 17: 1216.
Proskurnina, N.F. and A.P. Yakovlena. 1952. J. Gen. Chem. USSR 22: 1899; and 1953. Chem. Abst. 47: 6959c.
Rupreht, J., B. Dworacek, R. Ducardus, P.I.M. Schmitz, and M.R. Dzoljic. 1983. The involvement of the central cholinergic and endophinergic systems in the nitrous oxide withdrawal syndrome in mice. Anesthesiol. 58: 524-526.
Stoyanov, E.A. 1964a. Galanthamine hydrobromicum "Nivalin", ein neues Antidot der nichtpolarisierenden Muskelrelaxantien. Der Anaesthetist 13: 217.
Stoyanov, E.A. 1964b. Spirographic evidence on the anticurare effect of Nivalin. Acta Inst. Sup. Med. (Sofia) 43: 39.
Stoyanov, EA. and S. Vulchanova. 1963. The clinical application of Nivalin as an antidote of curare. Acta Inst. Sup. Med. (Sofia) 42: 45.
Stoyanov, E.A., H. Dobrev, and D. Paskov. 1965. The effect of Nivalin on respiration in steroid narcosis. Acta Inst. Sup. Med. (Sofia) 44: 1.
Stoyanov, E.A. and P. Statkov. 1968. Experimental study of the antagonism between fentanyl and Nivalin. Med. Arch. (Sophia) 6: 105.
Tassonyi, E., K. Labencz, I. Vimlati, and I. Kiss. 1976. Postoperative apnoea following Nivalin administration. Anaesthetist 25: 529-531.
Uyeo, S. and S. Kobayashi. 1953. Pharm. Bull. Tokyo 1: 139.
Westra, P., M.J.S. van Thiel, G.A. Vermeer, A.M. Soeterbroek, A.H.J. Scaf, and H.A. Claessens. 1986. Pharmacokinetics of galanthamine (a long-acting anticholinesterase drug) in anaesthetized patients. Br. J. Anaesth. 58: 1303-1307.
Williams, D.J. and D. Rogers. 1964. The structure of galanthamine methiodide. Proc. Chem. Soc.: 357.
Wislicki, L. 1967. Nivalin (galanthamine hydrobromide), an additional decurarizing agent. Some introductory observations. Br. J. Anaesth. 39: 963-968.

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EXHIBIT 32

REGULAR ARTICLES

Enhancement of Memory Processes in Alzheimer's Disease with Multiple-Dose Intravenous Physostigmine

BY KENNETH L. DAVIS, M.D., AND RICHARD C. MOHS, PH.D.

Physostigmine (.125 mg, .25 mg, or .50 mg) or placebo was administered intravenously to 10 neuroleptic-free patients with Alzheimer's disease over a 30-minute period. All patients performed better on a recognition memory task while receiving physostigmine. When placebo or the dose of physostigmine previously associated with an improvement in memory was readministered, physostigmine again enhanced performance on a recognition memory task. These results indicate that the acute augmentation of cholinergic activity in some patients with Alzheimer's disease can partially reverse the memory deficit of that disorder and may provide an approach to the eventual therapy of this condition. (Am J Psychiatry 139:1421–1424, 1982)

Recent studies have demonstrated that Alzheimer's disease, the most common cause of dementia among elderly people, is a disorder that impairs the functioning of cholinergic neurons. Patients with Alzheimer's disease have a dramatic loss of brain choline acetyltransferase (1–10), a marker for intact cholinergic neurons (11). The loss of brain choline acetyltransferase activity has been correlated with both the degree of dementia and the histopathological changes in the brain that are characteristic of Alzheimer's disease (8). On the basis of these findings and the fact that choline and phosphatidylcholine, precursors of acetylcholine, can increase acetylcholine concentrations in the brain (12–16), many studies have been conducted to investigate the effects of these precursors on memory in normal people and patients with Alzheimer's disease (17–28). Unfortunately, these studies have not convincingly demonstrated any reliable enhancement of memory after treatment with precursors of acetylcholine. Alternative methods for pharmacologically enhancing cholinergic activity include the use of cholinesterase inhibitors and cholinergic agonists. Both physostigmine, a short-acting cholinesterase inhibitor, and arecoline, a short-acting cholinergic agonist, have been shown to enhance storage of information into memory when given in low doses to healthy young adults (29, 30). We are now able to report that physostigmine also acutely enhanced memory when given, under double-blind conditions, to 10 patients with Alzheimer's disease.

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METHOD

The sample consisted of 8 male and 2 female patients between the ages of 50 and 68 years. The diagnosis of Alzheimer's disease was made with the aid of computerized tomographic scan, brain skull films, CSF analysis, serum analysis, a carefully taken history, and physical examination. Particular care was given to ruling out cases of multi-infarct dementia. All patients had a Memory and Information Test score of 10 or less and/or a Dementia Rating Scale score of 4 or more. These criteria have been shown to identify patients with a high probability of Alzheimer's disease, as verified by histopathological examination on autopsy (31). The patients had been free of all psychoactive agents for at least 2 weeks before physostigmine administration, with the exception of an occasional dose of chloral hydrate at bedtime. The patients were not psychotic or agitated and were able to cooperate with the cognitive testing procedures. In practice, the 2 previously mentioned criteria defined a rather homogeneous group of moderately demented but cooperative subjects.

Because of the unusual dose-response characteristics of physostigmine (29, 30), drug administration was divided into two phases. The first, or dose-response, phase was designed to determine the optimal dose for each patient. In this phase subjects received, under double-blind conditions, placebo or .125 mg, .25 mg, or .50 mg of physostigmine in a random order on separate days. The drug was dissolved in 100 cc of saline and administered at a constant rate for 30 min. In the second, or replication, phase of the study, the dose of

physostigmine previously associated with the best performance on cognitive tests involving storage of information into long-term memory was readministered, as was the placebo infusion. The order of these two infusions was also randomized, and the conditions of administration were double-blind. Two to 4 days generally separated each infusion, which always occurred at the same time of day. A dose of 2.5 mg of probanthine, a cholinergic antagonist that does not cross the blood-brain barrier, was administered intravenously 5 min before the start of every infusion to minimize physostigmine's peripheral effects.

Subjects' memory functioning was assessed by cognitive tasks administered in the following order: 1) Famous Faces Test (32), 2) Digit Span Task, and 3) Recognition Memory Test for either 12 words or 12 pictures (33–35). Approximately 1 week before the administration of physostigmine, baseline cognitive performance was assessed on two occasions. On drug-free days testing began after the infusion started and ended 10 min after the infusion stopped. The more demented subjects were assessed with the picture recognition task and the less demented subjects with the word recognition task. Three trials were completed on the picture or word recognition task. In the first, or study, phase of each trial, patients described each picture briefly or read each word. In the second, or test, phase, the 12 studied items were presented together with 12 similar words or pictures that had not been presented previously. The patient's task was to decide whether each of the 24 items had been presented previously.

These tests were selected to measure the subjects' ability to store information in long-term memory, to be sensitive to an improvement or worsening in performance, to be comprehensible to the subject, and to be completed in the time period of physostigmine's biological activity. The distinction between short-term and long-term memory is an essential feature of many current psychological theories of memory and is supported by studies of patients with hippocampal lesions. Short-term memory is presumed to be of limited capacity and can be measured in seconds. The Digit Span Task is a measure of short-term memory. Long-term memory is essentially of unlimited capacity and is where information is permanently stored. Learning a list of words involves the storage of information in long-term memory. The ability to recall a previously learned name measures retrieval from long-term memory (32–41).

TABLE 1. Recognition Memory in 10 Patients with Alzheimer's Disease During Dose-Response Phase of Physostigmine Treatment

	Mean Percent Correct on the Recognition Memory Test[a]			
		Physostigmine		
Patient	Placebo	.125 mg	.25 mg	.50 mg
1	75.0	60.4	76.4	87.5[b]
2	73.6	70.8	70.8	79.2[b]
3	61.1	66.7	70.8[b]	65.3
4	45.8	69.5[b]	62.5	55.6
5	59.7	81.9[b]	65.3	69.4
6	75.0	79.2	90.3[b]	88.9
7	84.7	80.6	86.1	88.9[b]
8	65.3	66.7[b]	63.9	61.1
9	80.6	77.8	80.5	87.5[b]
10	69.4	75.0	79.2	88.9[b]

[a]Each percent is the mean for 3 trials. Raw scores can be obtained by multiplying the mean percent of correct responses by 24. The number of errors can be obtained by subtracting the raw scores from 24.
[b]The patient's best response

TABLE 2. Recognition Memory in 10 Patients with Alzheimer's Disease During Replication Phase of Physostigmine Treatment

	Mean Percent Correct on the Recognition Memory Test		
Patient	Placebo	Physostigmine	Change
1	76.38	79.17	2.79
2	55.54	73.62	18.08
3	63.88	63.88	0.00
4	58.33	68.04	9.71
5	65.29	75.00	9.71
6	83.33	97.21	13.88
7	73.58	91.67	18.09
8	55.54	63.88	8.34
9	80.54	72.21	−8.33
10	72.21	75.00	2.79
Mean	68.47	75.97	7.50

RESULTS

Table 1 presents the results obtained from all 10 patients on the picture or word recognition task during the dose-response phase of the study. All patients had their best performance in ability to store information in long-term memory on some dose of physostigmine rather than on the placebo saline infusion. In all of these patients the best dose of physostigmine varied among .125 mg, .25 mg., and .50 mg. Although it was not possible to completely balance the order of drug doses, an analysis of variance performed on the memory test scores with test days as a repeated measures factor revealed no tendency for scores to change with repeated testing ($F=2.5$, $df=3, 27$, $p>.07$). Factors that might predict the dose of physostigmine most likely to enhance memory were not readily apparent, although the data in table 1 suggest that the best dose decreased as patients' ability to perform the task decreased.

The results of the replication phase of the study are presented in table 2. During this phase only 1 patient's performance was better during the saline than during the physostigmine infusion. Another patient had an equivalent performance during both infusions, and 8 patients demonstrated a physostigmine-related improvement in long-term memory storage. A paired t test indicated that this enhancement due to physostigmine was significant ($t=2.84$, $df=9$, $p<.01$, one-tailed). Baseline memory test scores differed by an average of 2%.

Two other statistical analyses were also performed on the data from the replication study. A mixed model analysis of variance was performed with order of drug and placebo administration as a between-subjects factor and with drug conditions (physostigmine versus placebo) and learning trials (1, 2, and 3) as orthogonal within-subjects factors. Of the 2 groups formed by considering order of drug administration, 1 consisted of 6 patients who received placebo first and the other consisted of 4 patients who received physostigmine first. The analysis revealed no effect due to order of drug administration and no significant interactions involving order of drug administration ($p>.10$ in all cases). There was, however, a significant increase in percent of correct responses over trials ($F=6.25$, $df=2, 16$, $p<.01$) and a significantly greater percent of

correct responses in the physostigmine condition ($F=6.92$, $df=1, 8$, $p<.03$). The interaction of trials with drug conditions was not significant ($p>.10$). Since it is possible that these data do not satisfy all of the assumptions required for parametric statistical analysis, the scores presented in table 2 were also analyzed by means of a nonparametric sign test. This test also demonstrated that the enhancement of memory due to physostigmine was statistically significant ($p<.02$, one-tailed).

Analysis of the data from the Digit Span Task, which measures the capacity of short-term memory, and the Famous Faces Test, which measures retrieval from long-term memory, indicated that physostigmine had no effect on performance of these tasks.

Baseline memory test scores obtained on the Recognition Memory Test on two occasions before the dose-response phase differed by 2%.

DISCUSSION

Low doses of intravenous physostigmine transiently improved the ability of patients with Alzheimer's disease to store information into long-term memory, as demonstrated by the Recognition Memory Test. This effect was demonstrated twice—in the dose-response phase of the study and again in the replication phase. This finding is consistent with similar effects of physostigmine and arecoline in young normal subjects (29, 30) and with a preliminary report of the effects of intravenous physostigmine on a small group of patients with Alzheimer's disease (42). Following that last report physostigmine, the muscarinic agonist arecoline, and the longer-acting acetylcholinesterase inhibitor tetra-hydroaminoacridine have been administered to a number of patients with Alzheimer's disease. In every instance in which multiple doses of a cholinomimetic agent were administered to a sample of patients with Alzheimer's disease, there was a beneficial response in a variable subgroup of patients. The ability to encode new information into long-term memory was enhanced in the majority of patients in two studies (43, 44) and to a lesser extent in another study (45). Administration of tetra-hydroaminoacridine produced a general global improvement in 9 of 12 patients but a more modest, although positive, effect in another series of patients (46). Physostigmine markedly enhanced a patient's constructional praxis (47) and diminished intrusion errors (48). There have been two negative reports encompassing very few patients with Alzheimer's disease. One tested the effect of a single dose of pilocarpine in a heterogeneous group of elderly people with dementia including Korsakoff's dementia (49). The other study, which investigated the effects of a single dose of intravenous physostigmine in patients with Alzheimer's disease (50), pointed out that in order to find a positive effect of physostigmine it may be critical that "the dose is titrated individually"; that study did not follow such a procedure.

An inevitable question in any study of cholinomimetic agents in Alzheimer's disease is the clinical significance of the drug's effect. In the present investigation the absolute magnitude of physostigmine's effect can be judged by comparison both with nondemented people and with the baseline variability of patients with Alzheimer's disease on recognition memory tests. Compared with nondemented people, the patients in this study were quite impaired even while receiving physostigmine. Baseline memory test scores differed by an average of 2%, considerably less than the drug's effect. Thus, the acute effect of physostigmine to enhance memory was larger and more consistent than the normal day-to-day fluctuation in memory test performance among these patients, even though they remained quite impaired compared with nondemented people. However, until there is long-term administration of cholinomimetic agents to patients with Alzheimer's disease, it will be impossible to judge their ultimate clinical utility.

In summary, these data support the hypothesis that cholinergic neurons are critically involved in the storage of information in long-term memory. Furthermore, they suggest that the cholinergic deficit found on neuropathological examination contributes to the cognitive changes in patients with Alzheimer's disease and that reversal of that deficit may provide an approach to the treatment of the disorder.

REFERENCES

1. Davies P, Maloney AJF: Selective loss of central cholinergic neurons in Alzheimer's disease (ltr to ed). Lancet 1:1403, 1976
2. Perry EK, Gibson PH, Blessed G, et al: Neurotransmitter abnormalities in senile dementia. J Neurol Sci 34:247–265, 1977
3. White P, Hiley CR, Goodhardt MJ: Neocortical cholinergic neurons in elderly people. Lancet 1:668–671, 1977
4. Reisine TD, Yamamura MI, Bird ED, et al: Pre and postsynaptic neurochemical alterations in Alzheimer's disease. Brain Res 159:477–481, 1978
5. Davies P: Biochemical changes in Alzheimer's disease—senile dementia, in Congenital and Acquired Cognitive Disorders. Edited by Katzman R. New York, Raven Press, 1979
6. Bowen DM, Spillane JA, Curzon G, et al: Accelerated ageing or selective neuronal loss as an important cause of dementia. Lancet 1:11–14, 1979
7. Perry EK, Perry RH, Blessed G, et al: Necropsy evidence of central cholinergic deficits in senile dementia (ltr to ed). Lancet 1:189, 1977
8. Perry EK, Tomlinson BE, Blessed G, et al: Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J 2:1457–1459, 1978
9. Sims CR, Bowen DM, Smith CCT, et al: Glucose metabolism and acetylcholine synthesis in relation to neuronal activity in Alzheimer's disease. Lancet 1:333–335, 1980
10. Davies P: Neurotransmitter-related enzymes in senile dementia of the Alzheimer type. Brain Res 171:319–327, 1979
11. Kuhar MH: The anatomy of cholinergic neurons, in Biology of Cholinergic Function. Edited by Goldberg AM, Hanin I. New York, Raven Press, 1976
12. Haubrich DR, Wang PFL, Wedeking P: Role of choline in biosynthesis of acetylcholine. Fed Proc 33:447, 1974
13. Cohen EL, Wurtman RJ: Brain acetylcholine: increase after

systemic choline administration. Life Sci 16:1095–1102, 1975
14. Ulus IH, Wurtman RJ: Choline administration: activation of tyrosine hydroxylase in dopaminergic neurons of rat brain. Science 194:1060–1061, 1976
15. Davis KL, Hollister LE, Barchas JD, et al: Choline in tardive dyskinesia and Huntington's disease. Life Sci 19:1507–1516, 1976
16. Growden JH, Hirsch MJ, Wurtman RJ, et al: Oral choline administration to patients with tardive dyskinesia. N Engl J Med 297:524–527, 1977
17. Boyd WD, Graham-White J, Blackwood G, et al: Clinical effects of choline in Alzheimer senile dementia (ltr to ed). Lancet 2:711, 1977
18. Etienne P, Gauthier S, Johnson G, et al: Clinical effects of choline in Alzheimer's disease. Lancet 1:508–509, 1978
19. Signoret JL, Whiteley AL, Lhermitte F: Influence of choline on amnesia in early Alzheimer's disease (ltr to ed). Lancet 2:837, 1978
20. Etienne P, Gauthier S, Dastoor D, et al: Lecithin in Alzheimer's disease (ltr to ed). Lancet 2:1206, 1978
21. Ferris SH, Sathanathan G, Reisberg B, et al: Long-term choline treatment of memory-impaired elderly patients. Science 205:1039–1040, 1979
22. Christie JE, Blackburn IM, Glen A, et al: Effects of choline and lecithin on CSF choline levels and cognitive function in patients with presenile dementia of the Alzheimer type, in Nutrition and the Brain, vol 5. Edited by Barbeau A, Growdon JH, Wurtman RJ. New York, Raven Press, 1979
23. Smith CM, Swash M, Exton-Smith AN, et al: Choline therapy in Alzheimer's disease (ltr to ed). Lancet 2:318, 1978
24. Renvoize EB, Jerram T: Choline in Alzheimer's disease (ltr to ed). N Engl J Med 301:330, 1979
25. Peters BM, Levin HS: Effects of physostigmine and lecithin on memory in Alzheimer's disease. Ann Neurol 6:219–221, 1979
26. Mohs RC, Davis KL, Tinklenberg JR, et al: Choline chloride treatment of memory deficits in the elderly. Am J Psychiatry 136:1275–1277, 1979
27. Mohs RC, Davis KL, Tinklenberg JR: Cognitive effects of physostigmine and choline chloride in normal subjects, in Brain Acetylcholine and Neuropsychiatric Disease. Edited by Davis KL, Berger PA. New York, Plenum Press, 1979
28. Mohs RC, Davis KL: Choline chloride effects on memory: correlation with the effects of physostigmine. Psychiatry Res 2:149–156, 1980
29. Davis KL, Mohs RC, Tinklenberg JR, et al: Physostigmine: improvement of long-term memory processes in normal humans. Science 201:272–274, 1978
30. Sitaram N, Weingartner H, Gillin JC: Human serial learning: enchancement with arecoline and impairment with performance on placebo. Science 201:274–276, 1978
31. Kay DWK: The epidemiology and identification of brain deficits in the elderly, in Cognitive and Emotional Disturbances in the Elderly. Edited by Eisdorfer C, Friedel RO. Chicago, Medical Book Publishers, 1977
32. Marlsen-Wilson WD, Teuber JL: Memory for remote events in anterograde amnesia: recognition of public figures from news photographs. Neuropsychologia 13:353–364, 1975
33. Buschke H: Selective reminding for analysis of memory and learning. Journal of Verbal Learning and Verbal Behavior 12:543–550, 1973
34. Buschke H, Fuld PA: Evaluating storage, retention and retrieval and disordered memory and learning. Neurology 24:1019–1025, 1974
35. Shepard RN: Recognition memory for words, sentences, and pictures. Journal of Verbal Learning and Verbal Behavior 6:156–163, 1967
36. Bartus RT: Physostigmine and recent memory: effects in young and aged nonhuman primates. Science 206:1087–1089, 1979
37. Anderson JR, Bower GH: Human Associative Memory. Washington, DC, VH Winston, 1973
38. Atkinson RC, Shiffin RM: The control of short-term memory Sci Am 224:82–90, 1971
39. Baddeley AD: The Psychology of Memory. New York, Basic Books, 1976
40. Milner B: Memory and the medial temporal regions of the brain, in The Biology of Memory. Edited by Pribram KH, Broadbent DE. New York, Academic Press, 1970
41. Warrington EK: An analysis of short-term and long-term memory deficits in man, in The Physiological Basis of Memory. Edited by Deutsch JA. New York, Academic Press, 1973
42. Davis KL, Mohs RC, Tinklenberg JR: Enhancement of memory by physostigmine. N Engl J Med 301:946–947, 1979
43. Christie JE, Shering A, Ferguson J, et al: Physostigmine and arecoline: effects of intravenous infusions. Br J Psychiatry 138:46–50, 1981
44. Peters BH, Levin HS: Effects of physostigmine and lecithin on memory in Alzheimer's disease. Ann Neurol 6:219–221, 1979
45. Sullivan EV, Shedlack KJ, Corkin S, et al: Physostigmine and lecithin in Alzheimer's disease, in Alzheimer's Disease: A Report of Progress in Research. Edited by Corkin S, Davis KL, Growdin JH, et al. New York, Raven Press, 1982
46. Kaye WH, Sitaram N, Weingartner H, et al: Modest facilitation of memory in dementia with combined lecithin and anticholinesterase therapy. Biol Psychiatry 17:271–274, 1982
47. Muramoto O, Sugishita M, Sugita H, et al: Effect of physostigmine on constructional and memory tasks in Alzheimer's disease. Arch Neurol 36:501–503, 1979
48. Smith CM, Swash M: Physostigmine in Alzheimer's disease (ltr to ed). Lancet 1:42, 1979
49. Ashford JW, Soldinger S, Schaeffer J, et al: Physostigmine and its effect on six patients with dementia. Am J Psychiatry 138:829–830, 1981
50. Caine ED: Cholinomimetic treatment fails to improve memory disorders. N Engl J Med 303:585–586, 1980

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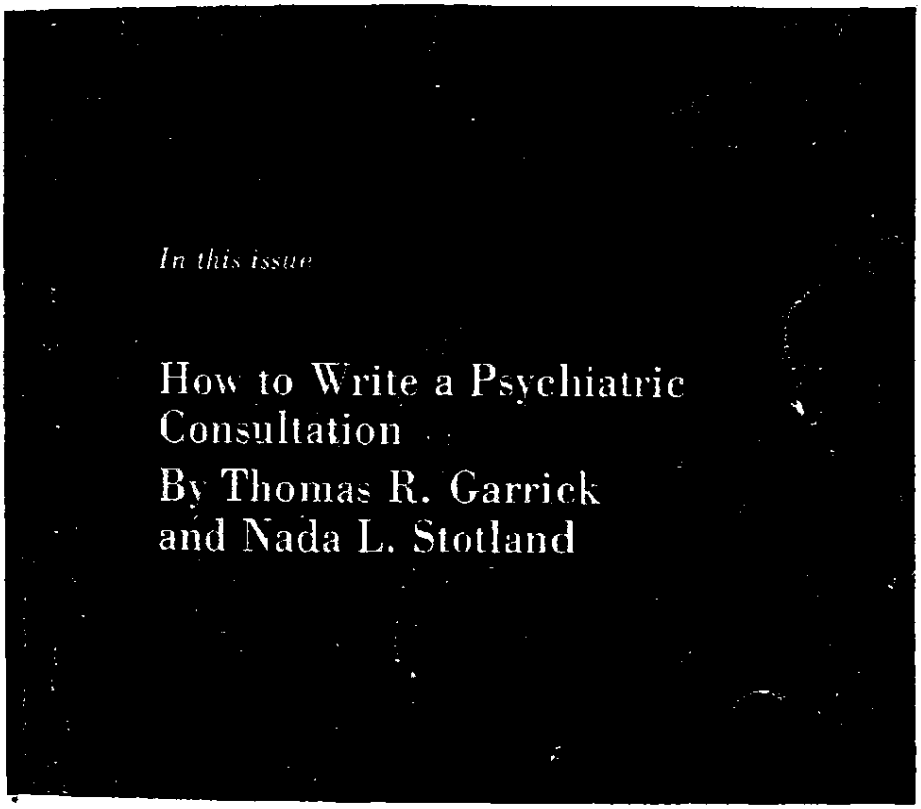

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EXHIBIT 38

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Anaesthesia, 1974, Volume 29, pages 163–168

In 8 given N-M block & general anesthesia, reversal was done = either a peripheral or peripheral + central anticholinesterase (neostigmine vs galanthamine) & atropine. The latter is to control. Their effect is: Ach — muscarinic block = nicotinestein.

Galanthamine hydrobromide versus neostigmine

A plasma cortisol study in man

Refs for ICD

2, 12, 16 — analeptic action of galanthamine
EEG ↑ in Ach ↑

D. A. COZANITIS

Galanthamine hydrobromide (Nivalin, Pharmachim, Sophia, Bulgaria), the result of Bulgarian and Russian work, is an anticholinesterase drug whose chemical structure closely resembles that of morphine (Fig. 1). Possession of a tertiary ammonium group enables the drug to traverse the blood–brain barrier, giving it central action in addition to its peripheral activity.

Naumenko, Ilyuchenko & Nesterenko[1] found that a subcutaneous injection of galanthamine hydrobromide caused an increase in 17-hydroxycorticosteroid blood levels in guinea-pigs and, in another study, conscious epileptic volunteers receiving

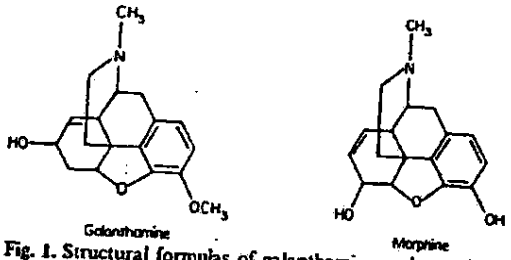

Fig. 1. Structural formulas of galanthamine and morphine.

an intravenous injection of this substance manifested a significant rise in peripheral plasma cortisol.[2]

The rise of plasma cortisol during anaesthesia has been attributed to the nature of the surgery rather than the anaesthetic agents.[3] The purpose of the present investigation was therefore to examine the effect of galanthamine hydrobromide on plasma cortisol in patients receiving relaxant anaesthesia. For purposes of comparison, a series of similar patients received neostigmine in place of galanthamine hydrobromide.

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Plaintiff's Exhibit PX-829

Method

The investigations were performed on twenty-three patients undergoing ligation and stripping of varicose veins. Some clinical details are noted in Table I. Only those patients without pre-existing heart or chest disease were included in the study. Attention was given to the nature of current drug therapy, if any, and particularly to those patients taking oestrogenic or thyroid medication. Patients on oral contraceptives were not included in the investigation.

The patients arrived early on the morning of their scheduled surgery, having fasted overnight and without any sedation on the night prior to their admission. Premedication consisted of atropine 0·01 mg/kg and pethidine 1 mg/kg injected intramuscularly 30 minutes before the induction of anaesthesia, which took place between 09.00 and 11.00 hours.

A pre-curarisation (3 mg) dose of tubocurarine[4] was given intravenously at the start of a 3-minute pre-oxygenation period and, during this period, 2·5% thiopentone (5 mg/kg) was administered intravenously. Suxamethonium (1·5 mg/kg) was injected intravenously after induction of sleep; the patient was then intubated and connected to a Manley ventilator delivering nitrous oxide and oxygen (5:2) according to the Adelaide nomogram.[5] A further dose of tubocurarine (0·5 mg/kg) was given followed by fentanyl 0·1 mg. Increments of fentanyl 0·05 mg were given as required when signs of sympathetic overactivity appeared (tachycardia, hypertension, sweating). The patients received all these drugs through a slow intravenous drip of physiological electrolyte solution.

The non-depolarising block was reversed at the end of surgery either with intravenous atropine 0·5 mg plus neostigmine 1 mg or atropine 0·5 mg plus galanthamine hydrobromide 20 mg. If more neostigmine proved to be necessary, the patient was

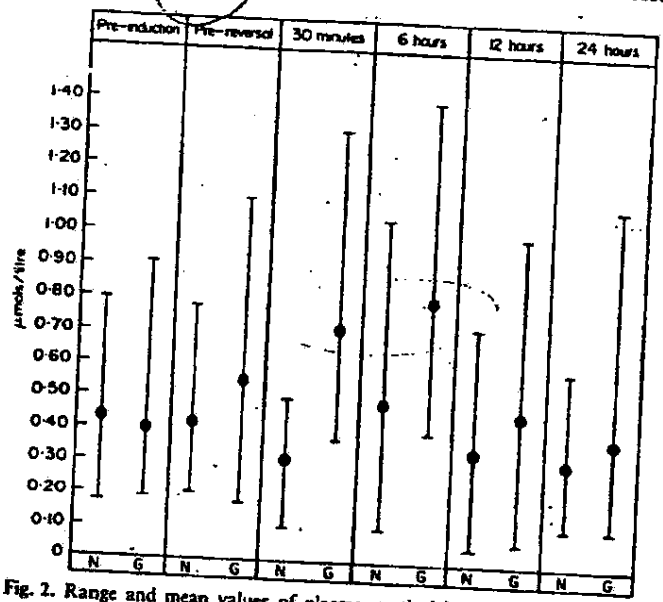

Fig. 2. Range and mean values of plasma cortisol before and after neostimine and galanthamine reversal. N, neostigmine; G, galanthamine.

excluded from the study. Ventilation with nitrous oxide and oxygen was maintained until after the injection of the reversal drugs.

Venous blood samples were taken in a heparinised tube immediately prior to the small tubocurarine dose, before reversal, 30 minutes later and then at 6, 12, and 24 hours. The blood was centrifuged within 30 minutes of collection and plasma stored at −20° until cortisol estimation could be carried out. Based on a technique described by Murphy,[6] an improved method for cortisol determination was employed,[7] all measurements were done in duplicate and high values further repeated.

Results

The range and mean values of plasma cortisol for the two series are shown in Fig. 2 and are given in detail in Table 1. The plasma cortisol values for both series remained

Table 1. Details of individual cases.

Patient	Age (years)	Weight (kg)	Anaesthesia (minutes)	Plasma control (μmol/l) Pre-induction	Pre-reversal	30 minutes	6 hours	12 hours	24 hours
Galanthamine hydrobromide series									
1	43	65	80	0·55	0·72	0·57	0·43	0·14	0·16
2	33	55	70	0·27	0·17	0·37	1·40	0·62	0·20
3	31	70	65	0·28	0·60	0·66	0·77	0·42	0·18
4	45	91	70	0·20	0·31	0·97	0·57	0·15	0·17
5	62	79	110	0·35	1·10	1·30	1·40	0·99	0·50
6	55	63	65	0·35	0·41	0·79	1·05	0·46	0·79
7	45	70	85	0·33	0·41	0·61	0·85	1·00	0·44
8	28	65	45	0·48	0·55	0·78	0·40	0·18	0·21
9	32	47	100	0·18	0·82	0·60	0·46	0·07	0·12
10	51	80	55	0·45	0·52	0·63	0·46	0·23	0·40
11	36	62	80	0·90	0·60	0·51	1·40	0·80	1·09
12	45	60	110	0·32	0·36	0·84	0·52	0·55	0·40
Mean	51	67	78	0·38	0·54	0·71	0·80	0·46	0·38
SE	±4·0	±3·4	±5·9	±0·07	±0·07	±0·11	±0·09	±0·08	±0·05
Neostigmine series									
1	46	85	125	0·35	0·77	0·28	1·04	0·33	—
2	49	53	90	0·61	0·46	0·38	0·67	0·72	0·59
3	52	60	85	0·79	0·70	0·47	0·24	0·26	0·12
4	33	91	105	0·27	0·20	0·10	0·39	0·40	0·29
5	55	57	100	0·26	0·24	0·16	0·54	0·51	—
6	43	87	115	0·17	0·30	0·17	0·10	—	—
7	28	70	90	0·55	0·24	0·33	0·84	0·33	0·31
8	38	60	70	0·44	0·50	0·41	0·51	0·39	0·37
9	41	62	80	0·30	0·44	0·49	0·59	0·05	0·29
10	51	53	55	0·46	0·30	0·28	0·15	0·10	0·25
11	28	60	60	0·45	0·44	0·40	0·35	0·42	0·43
Mean	42	67	87	0·42	0·41	0·31	0·49	0·35	0·33
SE	±3·0	±4·2	±6·6	±0·05	±0·05	±0·04	±0·08	±0·06	±0·04

within normal limits during anaesthesia, which ranged from 45 to 125 minutes (mean 83 minutes), and surgery for varicose veins. Thirty minutes after galanthamine hydrobromide a very significant ($t = 5{\cdot}050$, $P<0{\cdot}001$) rise in plasma cortisol was observed but, on the other hand, no significant change was seen following neostigmine administration. The cortisol level of the galanthamine hydrobromide series was still significantly raised 6 hours after administration ($t = 2{\cdot}164$, $0{\cdot}05>P>0{\cdot}02$).

One patient had been on thyroid-replacement therapy and a second on oestriol succinate for her menopausal state; neither showed abnormal cortisol levels prior to anaesthesia. A single patient exhibited a plasma cortisol value above normal before anaesthesia but this value had returned to practically the same pre-anaesthesia level 24 hours later.

Discussion

Clarke, Johnston & Sheridan[3] reported that the rise of plasma cortisol was related to the stress of surgery; they found that during surface surgery the plasma cortisol level rose slightly but during intra-abdominal surgical procedures the levels were more than double the resting values. No significant rises in plasma cortisol was observed in the present investigation during anaesthesia and surgery probably because only patients undergoing ligation and stripping of varicose veins were included and, except for two cases, surgery was performed on one leg only. The additional increments of the analgesic fentanyl may also have contributed to avoidance of cortisol rise during operation.

Relatively few drugs employed in anaesthesia provoke an elevation of plasma cortisol. Oyama,[8] in a review of endocrine responses to anaesthetic agents, states that diethyl ether and cyclopropane raise peripheral blood levels of corticoids as do some newer agents, particularly when used in combination. These include ketamine when administered with nitrous oxide and droperidol given with pentazocine.

The equipotent dose of neostigmine 1 mg and galanthamine hydrobromide 20 mg used in the present study is based on recent findings by Baraka & Cozanitis[9] studying the reversal of non-depolarising block. The clinical equipotent dose suggested by Mayrhofer[10] is, however, neostigmine 2 mg and galanthamine hydrobromide 20 mg.

The variations may arise because of the different pharmacology of the two agents. Galanthamine hydrobromide displays central activity as well as peripheral action. Wislicki,[11] who suggested an equipotency in the same ratio as Mayrhofer, described this central activity; she reported that adequate tidal volumes and wakefulness were not always associated with return of grip strength and ability to lift the head. More recent and specific studies[2,12] have shown that galanthamine hydrobromide is an analeptic drug and may be used to stimulate depressed respiration.

In contrast to earlier findings,[10] evidence of possible cardiac arrhythmias has been seen following galanthamine hydrobromide when atropine was omitted.[13] On the basis of this finding, the patients in this study received an intravenous injection of atropine 0·5 mg just before galanthamine hydrobromide was administered.

One patient in the series was receiving thyroid therapy. The rate of disappearance of cortisol from plasma is slower in patients with diminished thyroid function.[14] In the case of this particular patient, the pre-anaesthesia cortisol value was within normal limits; she was most probably euthyroid due to supplemental thyroid intake.

The patient receiving oestriol succinate also had a normal cortisol level; this agrees with findings by Leutscher & Cheville.[14]

Competitive protein-binding was chosen for plasma cortisol analysis for two reasons; firstly, this technique is more specific than fluorometry in that no non-steroidal substance has been shown to affect it, and only a few steroids are able to compete effectively for binding sites[15] and, secondly, a minimum (0·1 ml) of plasma is required producing less stress to the donor–patient.

This investigation provides evidence that galanthamine hydrobromide provokes a rise in plasma cortisol. This confirms the findings of Naumenko *et al.*[1] These Russian workers suggested that the rise of 17-hydroxycorticosteroids in guinea-pigs is due to the action of this compound on the pituitary–supra-renal system mediated by stimulation of peripheral cholinergic nerves. If it is remembered that neostigmine does not penetrate the blood–brain barrier, the analeptic action of galanthamine hydrobromide with stress and excitement, mild as it is,[16] might account for the elevation of plasma cortisol. This analeptic property is certainly not an undesirable quality and clinical experience with galanthamine hydrobromide has shown that patients in the post-operative period do not appear to require more analgesia than those having received neostigmine.

Summary

Few agents used in anaesthesia are known to provoke a rise of plasma cortisol. Galanthamine hydrobromide, an anticholinesterase drug, is capable of penetrating the blood–brain barrier. This substance given to reverse a non-depolarising neuromuscular block is shown to increase plasma cortisol above normal limits.

Acknowledgments

The author would like to thank Dr Ulla Aromaa and Dr Marja Mäkikuutti of the Department of Anaesthetics, Helsinki University Central Hospital, for their assistance and co-operation, Dr A. Dessypris of the Minerva Institute for Clinical Research, Kauniainen, Finland, for his guidance and advice, and Nurse Asta Laukkanen for her work in gathering patient information, which the author greatly appreciated. The help, encouragement, and criticisms of Dr Richard S. J. Clarke, Queens University of Belfast, were also most welcome. Thanks are also due to Pharmachim, Sophia, Bulgaria, for their very generous gift of Nivalin.

References

1. Naumenko, E.V., Ilyuchenko, R.I. & Nesterenko, L.N. (1965) On the mode of action of Nivalin upon the adreno-hypophyseal system (translated title). *Farmacologia i Toksikologia*, 28, 659.
2. Cozanitis, D., Toivakka, E. & Dessypris, A. (1973) Electroencephalographic and blood chemistry responses to galanthamine hydrobromide in epileptic volunteers. *Der Anaesthesist*, 22, 31.
3. Clarke, R.S.J., Johnston, H. & Sheridan, B. (1970) The influence of anaesthesia and surgery on plasma cortisol, insulin and free fatty acids. *British Journal of Anaesthesia*, 42, 295.
4. Mayrhofer, O. (1959) Die Wirksamkeit von D-Tubocurarin zur Verhütung der Muskelschmerzen nach Succinylcholin. *Der Anaesthesist*, 8, 313.
5. Kenny, S. (1967) The Adelaide ventilation guide. *British Journal of Anaesthesia*, 39, 21.

6. Murphy, B.E.P. (1964) Application of the property of protein-binding to the assay of minute quantities of hormones and other substances. *Nature*, **201**, 679.
7. Leclerq, R., Copinschi, G., Bruno, O.D. & Frankson, J.R.M. (1969) Dosage par compétition du cortisol plasmatique. *Fonds de la Recherche Scientifique Médicale* (*Bruxelles*), *Groupes de Contact*, **7**.
8. Oyama, T. (1973) Endocrine responses to anaesthetic agents. *British Journal of Anaesthesia*, **45**, 276.
9. Baraka, A. & Cozanitis, D. (1973) Galanthamine versus neostigmine for reversal of non-depolarizing neuromuscular block in man. *Anesthesia and Analgesia: Current Researches*, **52**, 832.
10. Mayrhofer, O. (1966) Clinical experiences with diallyl-nortoxiferine and the curare antidote galanthamine. *Southern Medical Journal*, **59**, 1364.
11. Wielicki, L. (1967) Nivalin (galanthamine hydrobromide): an additional decurarizing agent: some introductory observations. *British Journal of Anaesthesia*, **39**, 963.
12. Cozanitis, D.A., Rosenberg, P. & Toivakka, E. (1974) Treatment of respiratory depression with an anticholinesterase drug: galanthamine hydrobromide. A case report supplemented with animal experiments (in preparation).
13. Cozanitis, D.A., Nuuttila, K., Karhunen, P. & Baraka, A. (1973) Changes in cardiac rhythm with galanthamine hydrobromide. *Der Anaesthesist*, **22**, 457.
14. Luetscher, J.A. & Cheville, R.A. (1968) In: *Clinical Endocrinology*. II. (Ed. by E.B. Astwood and C.E. Cassidy), p. 444. Grune and Stratton, New York.
15. Murphy, B.E.P. (1968) In: *Clinical Endocrinology*. II. (Ed. by E.B. Astwood and C.E. Cassidy), p. 456. Grune and Stratton, New York.
16. Cozanitis, D.A. & Toivakka, E. (1971) A comparative study of galanthamine hydrobromide and atropine/neostigmine in conscious volunteers. *Der Anaesthesist*, **20**, 416.

EXHIBIT 39

Attorney Client Communication
REDACTED
Attorney Client Communication

ORIGINAL INCOMING FAX

ciba

PHARMACEUTICAL DIVISION
DRUG DEVELOPMENT DEPARTMENT
556 MORRIS AVENUE
SUMMIT, N.J. 07901

LP - N.Y.
MAR 15 1994
RECEIVED

MARCH 14, 1994

TO:	Dr. Bonnie Davis	PHONE:	(516) 351-9192
COMPANY:	Intelligen Corp.	FAX:	(516) 423-3199
FROM:	Meg Stahl	PHONE:	(908) 277-5682
	(for Richard Katz, Ph.D.)	FAX:	(908) 277-4941

TOTAL PAGES: 5

COMMENTS: GALANTHAMINE PROTOCOL 01

Dear Dr. Davis:

Attached please find copies of the letters regarding the discontinuation of galanthamine development by Ciba and providing a synopsis of findings from the trial that were sent to all investigators working on Galanthamine Protocol 01. Please let me know if you need any other information from our files.

Sincerely,

Margaret M. Stahl

Margaret M. Stahl

Plaintiff's Exhibit
PX - 833

REDACTED
Attorney Client Communication

ciba

Pharmaceuticals Division

Ciba-Geigy Corporation
Summit, New Jersey 07901

December 16, 1993

Dr. F. Dal Bianco
Universitaetsklinik
Neurologie - Ambulanz
Wahringerguertel 18 - 20
1090 Wien
Austria

Re: Galanthamine Protocol 01 M6926Z

Dear Dr. Dal Bianco:

With some regret I want to inform you of Ciba's decision to discontinue the further development of galanthamine in the indication of primary degenerative dementia (Alzheimer's Disease). The management board of Ciba reached this decision without having any doubts about either the efficacy or the safety of galanthamine. This was a business decision reflecting limited development resources - it was well understood that galanthamine, according to the results of Protocol 01, promises to be an efficacious and, in comparison to other treatments, a safe drug.

Following this decision to discontinue the further development, all rights to investigate galanthamine were returned to the owner of the patent:

Dr. Bonnie Davis
Intelligen Corporation
P. O. Box 157
Cold Springs Harbor, NY 11724

Telephone: 516-423-3182
Fax: 516-423-3199

With receipt of this letter, Ciba is not anymore entitled to provide you with any information on galanthamine. If you wish to obtain additional data on the results of Galanthamine Protocol 01, please contact Dr. B. Davis directly.

MAR 14 '94 13:43 FROM DEVELOPMENT-CNS TO 915164233199 PAGE.003

I want to thank you for your cooperation and enthusiasm in performing Galanthamine Protocol 01.

With the best wishes for a Merry Christmas and a Happy New Year,

Sincerely yours,

Ursula Hartung

Ursula Hartung, Ph.D.
Associate Director
Development/CNS

UH:mms
invterm.ltr
c: Dr. R. Katz
Mr. R. Gerber
PSRC/F/P

ciba

Pharmaceuticals Division

Ciba-Geigy Corporation
Summit, New Jersey 07901

January 13, 1994

Professor Dr. E. Deisenhammer
Wagner Jauregg Krankenhaus
Wagner Jauregg Weg 15
4020 Linz
Austria

Dear Dr. Deisenhammer:

We are grateful for your efforts in support of Galanthamine Protocol 01, and are pleased to provide a synopsis of findings. As noted in the protocol we support your efforts to provide these data to a broader scientific community. Clearly the fairest and most representative presentation will include data from all sites. Dr. Davis has expressed a willingness to coordinate publication efforts, and interested parties should contact her directly at Intelligen Corporation, P.O. Box 157, Cold Springs Harbor, NY 11724.

Overall Findings: Findings from the two primary variables are provided. the ADAS Cog declined approximately 5 points during initial single blind dose escalation (Table 8.1.3.B.). Subsequently this measure (Tables 8.1.1.- 8.1.3.A.) demonstrated a directionally appropriate decline of slightly more than 2 points vs placebo at Visit T. (Under Galanthamine an improvement of about 0.8 and under placebo a deterioration of about 1.3). This change was not significant statistically, between groups, although Visit 11 and 12 within - patient data do indicate significant changes for galanthamine treated patients vs. their own baseline.

The Physician's Global Scale (Tables 8.1.2; 8.1.7.A. - C.) was significant between - groups from V. 11, and inclusive of Visit T. Changes across these two primary measures tended to covary (Fig 2).

Secondary measures tended to be consistent with the above findings, however per the statistical protocol these measures were summarized without inferential statistics (ADAS non Cog = Table 8.1.4; Axis V Table 8.1.6; QLS = 8.1.9 MMSQ = Table 8.1.10

Findings support the therapeutic potential of galanthamine in Alzheimer's Disease.

MAR 14 194 13:44 FROM DELEGEMENT-CHS TO 01516 [illegible] PAGE 002

The following by-center information specific to each investigational site is provided

Table 7.1.1. demographics
Tables 8.1.1A, 8.1.1B - ADAS Cog Globals
Table 8.1.C A,E Physician's Global
Data listing V1.1 - randomization codes

Our thanks again for your participation and efforts, throughout.

Cordially,

Richard Katz, Ph.D.
Director
CNS/Development

Ursula Hartung, Ph.D.
Associate Director
CNS/Development

cc: Dr. C. Lee

RK:llw
(galinv.var)

Due regard shall be given to CIBA-GEIGY's legitimate interests, e.g., obtaining optimal patent protection, coordinating and maintaining the proprietary nature of submissions to health authorities, coordination with other ongoing studies in the same field, and protection of confidential data and information.

CIBA-GEIGY'S comments shall be given without undue delay. If they are not accepted, the senior author of the manuscript and the CIBA-GEIGY representative(s) shall promptly meet to further discuss and endeavor to mutually agree on the final wording and/or disposition of the publication.

The above procedure also applies to information on prematurely discontinued and other non-completed studies.

Results from investigations shall not be made available to any third party by the investigating team outside the publication procedure as set out above.

CIBA-GEIGY Corporation will not quote from publications by investigators in its scientific information and/or promotional material without full acknowledgment of the source (i.e., author and reference).

All publications must indicate that CIBA-GEIGY was the source of galanthamine.

TARGET DATES

Onset of trial: _____

Conclusion of trial: _____

APPROVED BY

_____ _____
Investigator Date

_____ _____
Monitor Date

(GAL.P01:12/20/90)

EXHIBIT 40

17 Seacrest Drive
Huntington, New York 11743
Phone: (516) 423 3182
Fax: (516) 423 3199
February 24, 1989

Dr. S. Enna
Nova Pharmaceutical Corporation
6200 Freeport Centre
Baltimore, Maryland 21224

Dear Dr. Enna,

It is a pleasure that a person of your academic reputation is interested in galanthamine.

The standard documents,"Galanthamine for Alzheimer's Disease and Related Dementias," and "Galanthamine Toxicity," summarize information relevant to galanthamine's use in Alzheimer's, and the primarily English-language literature on galanthamine safety and tolerability. The third one, "Galanthamine Analogs, Structure/Function Relationships," can be sent pending a ten-year confidentiality agreement. My attorney thinks galanthamine could be known to be efficacious in fewer than five years, at which point the analog case could still be under consideration by certain backlogged foreign patent offices.

The analog patent applications also specify a combination product containing galanthamine-type compounds and noradrenergic drugs. This should meet the needs of more advanced patients with locus coeruleus as well as basalis lesions.

Enclosed also are a recent publication, submission and poster from Joe Coyle's lab.

In answer to your question of what evidence there is for a therapeutic effect in AD, there isn't any. There are three enclosures which contain clues. They provide evidence that galanthamine has CNS activity in humans. Two old anesthesia papers describe Laborit-type observations. Stojanov and Wislicki are struck by the rapidity of wakefulness and responsivity occurring in anesthetized patients following galanthamine administration for the reversal of neuromuscular blockade. This is noted in contrast to other cholinesterase inhibitors, and to occur when peripheral cholinesterase inhibition, as evidenced by continued muscular weakness, is not well established.

Stimulating consciousness in the intact brain is not equivalent to reversing the cholinergic deficiency in Alzheimer's disease, and many substances which may be cognitive enhancers in normals have failed in Alzheimer's trials. These observations merely indicate that galanthamine rapidly partitions to and exerts notable activity in the brain in humans.

The Baraka article demonstrates galanthamine reversal of scopolamine dementia in human volunteers. Given the loss of nicotinic as well as muscarinic agonism resulting from the ACh deficiency in Alzheimer's, and the failure of muscarinic agonists to have significant cognitive effects, I wouldn't take scopolamine dementia to be a model of Alzheimer's disease. This paper simply provides further evidence that galanthamine has potent CNS activity in humans.

The answer to your question about peripheral cholinergic side effects is that they could occur, but are unlikely to be prominent, and there are ways of getting around them. Galanthamine partitions to brain six-fold more strongly than physostigmine. The incidence of side effects in the literature is low, and these studies administered the full daily dose at one or at most two times. Vincent (see bibliography) demonstrated cognitive effects for five days following a dose of galanthamine in mice. If galanthamine's effects in brain are so prolonged, then qid or tid dosing, or the sustained release forms specified in the patent, should be able to produce even lower incidences of side effects than have been described in the literature.

As much safety information as can be gleaned is in "Galanthamine Toxicity." At least two thousand patients have received the drug for periods of several weeks to six months, and had very good clinical tolerance. Had this been THA, 100-600 would have turned yellow, and 120-200, red. If galanthamine can be brought to the FDA when THA, or an aminoacridine are the only alternatives, the FDA will have to license something, and galanthamine will probably look much safer.

Galanthamine studies should proceed about twice as fast as THA studies, given THA's toxicity dropout rate. Recruitment may be easier, as patients prefer a safer drug.

I have attempted to provide information, if not answers, in response to your questions on the telephone. If you have further questions or comments, please phone or fax. The fax is also good for leaving a message if I am out.

Thank you for your consideration.

Yours truly,

Bonnie M. Davis, MD

Bonnie M. Davis, M.D.

EXHIBIT 41

AT

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
PERIPHERAL AND CENTRAL NERVOUS SYSTEM DRUGS
ADVISORY PANEL

ANTIDEMENTIA DRUG ASSESSMENT SYMPOSIUM

Volume I

9 o'clock a.m.

Thursday, June 15, 1989

Conference Rooms D&E
Parklawn Building
Rockville, Maryland

MILLER REPORTING CO., INC.
507 C Street, N.E.
Washington, D.C. 20002
(202) 546-6666

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Thomas Crook, Ph.D., Memory Assessment Clinics
Kenneth L. Davis, M.D., Mount Sinai School of Medicine
David A. Drachman, M.D., University of Massachusetts Medical School
Steven H. Ferris, Ph.D., New York University Medical School
Marshal Folstein, M.D., Johns Hopkins Hospital
Elkan Gamzu, Ph.D., Parke-Davis Division, Warner Lambert Company
Gilbert Honigfeld, PhD., Sandoz Pharmaceutical Corporation
Zaven Khachaturian, Ph.D., National Institute on Aging
Richard C. Mohs, Ph.D., Mount Sinai School of Medicine
Peter V. Rabins, M.D., Johns Hopkins Hospital
Allen Raskin, Ph.D., University of Maryland School of Medicine
Murray Raskind, M.D., University of Washington
Barry Reisberg, M.D., New York University Medical Center
Leon J. Thal, M.D., San Diego V.A. Hospital Medical Center
Herbert J. Weingartner, Ph.D., George Washington University
Peter J. Whitehouse, M.D., PhD., University Hospitals of Cleveland
Richard J. Wurtman, M.D., Massachusetts Institute of Technology

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Leon J. Thal, M.D., San Diego V.A. Hospital Medical Center
Herbert J. Weingartner, Ph.D., George Washington University

C O N T E N T S

MORNING PROCEEDINGS

DR. LEBER: I think the time has come. I am Paul Leber. I am Director of FDA's Division of Neuropharmacological Drug Products. On behalf of the Food and Drug Administration, and its Peripheral and Central Nervous System Drugs Advisory Committee, I welcome you to FDA's Antidementia Drug Assessment Symposium.

At the outset, I would like to recognize publicly the really extraordinary efforts of our staff who got together and contributed to the organization and planning of this meeting. Some of the thanks, of course, are due to the members of the Advisory Committee who helped in the planning and organization of the symposium.

Of course, the obvious; without a faculty, there couldn't be a symposium. Thus, each of us in this room, regulated industry, agency or academician, is in the debt of the eighteen prominent individuals, neuroscientists, physicians and investigators, who agreed to make the effort and take the time to come to Rockville and participate in this symposium.

Now, I would like to name them. They are Marilyn Albert of the Massachusetts General Hospital, Thomas Crook of Memory Assessment Clinics, Kenneth Davis of Mt. Sinai in New York, David Drachman of the University of Massachusetts, Steven Ferris of New York University, Marshall Folstein of

Johns Hopkins, Elkan Gamzu of Parke Davis, Gilbert Honigfeld of Sandoz, Zaven Khachaturian of the National Institutes of Aging, Richard Mohs of Mt. Sinai, Peter Rabins of the Johns Hopkins, Allen Raskin of the University of Maryland, Murray Raskind of the University of Washington, Barry Reisberg of New York University, Leon Thal of the San Diego V.A. who is also chair of the Advisory Committee, Herb Weingartner of George Washington University, Peter Whitehouse of the University Hospitals of Cleveland and Richard Wurtman of MIT.

Because time is short, I have several technical and procedural matters to cover. I will not review the professional accomplishments and credentials of our faculty individually. Suffice it to say that all have outstanding reputations, deservedly so, and each is an expert in the area of medicine, neuroscience or clinical investigation relevant to the topics that will be discussed in this symposium.

Now, a brief explanation of what we are going to do here this morning. Each of the eight sessions is going to begin with a brief two-to-three minute introduction given either by myself or Dr. Katz. The purpose of the introduction is largely to introduce the speaker and to highlight some of the regulatory goals and purposes of the session.

Next, a keynote speaker will make a fifteen to twenty-minute presentation, with or without slides, and at it's completion, the panel will assemble up here, approxi-

mately nine members plus the speakers plus Dr. Thal and either Dr. Katz or myself, the last two, who will be co-chairing the session.

Then we will enter into a period of approximately an hour of general discussion. The hope is that there will be much to discuss. So it is possible that we won't actually get through in time.

If we have time, however, we will gladly open the session to have comments from the members of the Advisory Committee who are not on the symposium faculty, per se, but are present at this meeting.

Finally, if additional time permits, at the discretion of the co-chairs, we will, in fact, take questions from the audience, at least for the allotted time of that session.

Some technical issues related to the Advisory Committee, per se; because the symposium is being held as a meeting of the PCNS, we have to set time aside, and probably should, anyway, to hear comments and opinions from citizens who are in the audience. Ordinarily, this type of open session is held at the beginning of an advisory committee, but because we have no formal vote before the Committee today, and because the symposium has to run according to a fixed schedule, we have decided to postpone those open sessions to the end of the day. They will follow the four

sessions on each day and go for as long as an hour, but probably no more.

Also, because this is an advisory committee meeting, I have to make the following statement for the official record, which relates to conflicts of interest: all grants, contracts and other financial interests in firms regulated by the Center for Drug Evaluation and Review which have been reported by participating Advisory Committee members present no potential for the appearance of a conflict of interest.

Now, another role I like: the housekeeping announcements. As you know, this is a smoke-free building. Please do not smoke in it. Two, as much as you may be caffeine-dependant, you may not bring food or beverages into the rooms. I apologize for that, but those are the house rules. If any of you are desperate, there is a cafeteria on this floor which is not easily-accessible. You have to go around either end of the block of rooms which block you from it.

Finally, I am going to ask Dr. Thal to do a very important technical maneuver. As Chair of the Advisory Committee, he is obliged to officially open the meeting. Dr. Thal, can I ask you to do that.

DR. THAL: Good morning. Welcome. I am glad you are all here. The twenty-first meeting of the PCNS is now in session. The following members of the PCNS are in attendance

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in this meeting: Dr. Anne Ingers, Dr. Roger Machel, Dr. Richard McQuillan, Dr. Steven Ferris, Dr. Boris Fern, Dr. Fred Butler and Dr. Herb Weingarten.

The session is now open, and I am going to call Dr. Leber to begin.

DR. LEBER: Thank you. Good morning, officially, and welcome to the symposium. Some background; over the course of this century, the life expectancy of each successive generation has increased steadily. As a result, a proportion of elderly, roughly defined as individuals over 65 in our population, has grown steadily from less than 4 percent at the turn of the century to, perhaps, more than 11 percent.

Unfortunately, because the incidence of Alzheimer's dementia increases disproportionately with increasing age, longer survival has been accompanied by a dramatic increase in the prevalence of dementia. Estimates of the prevalence vary depending upon the case definition employed and the epidemiologic techniques of identification used, but as many as 2 to 3 million Americans, perhaps more, may currently suffer from dementia.

This, while the individual born today can look forward to a longer life than his or her forbearers, he or she is also at the risk of falling victim to a relentless, devastating and tragic illness, an illness that destroys the very essence of personality and mental life, an illness that

renders its victims helpless and dependent, and an illness for which there is, currently, no prevention and, certainly, no cure.

The victims of Alzheimer's, as most in this room know, are not limited to those directly afflicted by its neurodegenerative pathologies. Family, loved ones, those who care and provide support for the Alzheimer's patient also suffer greatly and critically for extended intervals of time. Remember, beyond the anguish of watching a loved one become a helpless remnant of his or her former self, the caregiver runs the personal risk of physical and financial exhaustion.

Society, too, is also very much involved. The out-of-pocket and opportunity costs linked to Alzheimer's are enormous and growing, placing an ever-increasing demand on our public-health resources.

Little wonder, given this very dismal picture, that so many of us are interested in finding a cure or at least a treatment for Alzheimer's.

Everyone's greatest hope, of course, is for the discover of a means of primary prevention because, once the mind is lost, it is, perhaps, arguable whether the battle for the body is worth winning. That is not meant to dismiss the value of treatments that can arrest or retard the rate of progression of Alzheimer's, a illness which, importantly, has a course measured in years and may, often, extend for more

than a decade.

To the contrary, at this point in time, even a safe and effective symptomatic treatment for some cardinal sign and symptom of Alzheimer's would constitute a substantive therapeutic advance.

I want to emphasize this last point for both medical, ethical and regulatory purposes. I setting goals and expectations for the treatment of Alzheimer's that we all hope to see developed, we would all do well to remember that we, unless we are terribly lucky, have to settle for very modest therapeutic gains, at least for the moment.

Indeed, as we search for this cure of Alzheimer's, we would all do well to consider the old French aphorism which advises physicians about the limitations in their powers to heal. In rough translation, the lines warn practitioners what to expect of their treatments; rarely to cure, sometimes to comfort, but always to console.

In developing drugs for Alzheimer's, I think we should keep this humbling admonition in mind.

Importantly, the Federal drug regulatory laws and policies are very consistent with this kind of counsel. Neither Federal law nor regulation set a minimum average treatment effect size that must be met before a drug can be approved. The size of a treatment effect, if it can be measured at all in a meaningful way -- and that, in itself,

is a very interesting arguable question -- is only important in so far as it can be considered in the risk-to-benefit assessment that controls new drug approval.

In any case, whatever our individual expectations and standards for meaningful antidementia therapies, there is a clear societal consensus that it is in the common interest to speed the development of safe and effective treatments for Alzheimer's. Accordingly, the Federal government is very much involved and committed to this purpose.

However, the role taken by different Federal agencies and organizations can vary considerably. Some parts of the Federal Government, as most of you know, are primarily involved in efforts to identify the cause or causes of Alzheimer's and in attempts to unravel its pathogenesis. Although much of this work is not directed at the development of new drugs, per se, the information developed in such basic research is essential because it serves as a scientific basis for subsequent development of rational pharmacological treatment strategies.

Now, the NINDS, the NIA and NIMH, in particular, have extensive intra and extramural programs directed at furthering our understanding of Alzheimer's. To a lesser extent, these same agencies, acting on their own or through grant and contract support to investigators, are also involved in exploratory, or what we would call early clinical,

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testing of new investigational drug treatments.

However, important, and as most of you in this audience clearly know, it is private industry that ordinarily undertakes the task of developing and testing any new investigational drug that is a legitimate candidate for commercial distribution whether it is a prescription or OTC drug.

In short, it is not the Government but private industry and free-market forces that determine which drug products are selected for commercial development.

Now, as many in the audience also know, the FDA plays another role in drug development. Unlike the Federal agencies named so far, the FDA is not involved in the discovery of new drugs. It serves as administrator and monitor of the various clinical testing and evaluation procedures that Congress -- and I emphasize Congress -- determines new drugs must successfully negotiate before they are marketed.

The Congressional requirements for societal, pre-market clearance of new drugs, reflects a consensus that has developed over the course of this entire century. The basic requirements of this consensus are enumerated in the Federal law of the Federal Food, Drug and Cosmetic Act, but only in a very broad way.

As specified in the Act, before the FDA has the

right to allow a drug to enter the domestic American marketplace, its sponsor must submit an NDA which presents probative evidence from a comprehensive battery of tests to show, among other things, that the drug is chemically what it claims to be, that it is free of contaminants -- that is, pure -- that it is reliably bioavailable, performs well, that it is accurately and truthfully and comprehensively labeled, and, most critically, that it is both safe in use, effective in use, for the indications claimed in its labeling under the conditions of use; that is, according to the directions given for its use in its labeling.

That is a mouthful, but it was an important part of the regulatory strategy.

As noted earlier, the testing programs employed to develop new drugs are ordinarily planned and undertaken by private industry, not by the Federal Government and its agencies and, in particular, not by the Food and Drug Administration, a point often forgotten by much of society. The FDA, however, does attempt to provide detailed guidance to those regulated -- that means, the regulated industries -- about how the Agency intends to interpret the broadly-stated requirements of the law, the Federal Food, Drug and Cosmetic Act.

Thus, through a process of formal Notice and Comment Rulemaking, regulations are promulgated to inform

prospective sponsors about the types and numbers of tests that the Agency ordinarily expects will be successfully completed before marketing approval is granted.

These requirements, obviously, apply more or less uniformly to all drugs regardless of their pharmacologic class.

Obviously, too, details of specific requirements must, logically, be allowed to vary among different drug classes. An obvious example, the nature and amount of evidence required to establish that some inert noble gas will be safe and effective for use in general anesthesia differs considerably from that needed to establish that a novel heterocyclic chemical with nonlinear metabolism with several active metabolites, known toxicities and a potential for aversion and abuse will be safe and effective as a treatment for anxiety.

There are just going to be differences in those requirements.

Clearly, the outcome assessment measures employed, the skills and training of the investigators, the nature of the patient samples, the type of clinical trial design, the type of lab testing, the kind of physicals, the extent of special testing and the extent of the period of observation and numbers of patients needed to document safety in use are going to vary dramatically between these very different drug

classes.

Consequently, it is not unreasonable to expect that the Agency will enumerate specific sets of rules to explain how sponsors should go forward in each of these drug classes. So the Agency has, publishing drug product development guidelines for a variety of drug products.

It hasn't, however, ever issued any formal guidelines in drugs for dementia. The explanation of why it has not is very important and is a major reason that we had for organizing this particular symposium.

To begin, typical drug development guidelines are little more than summaries of what has worked in the past. They are fundamentally empirical, experientially-based, documents. For example, the Agency's antidepressant drug guidelines developed basically by ACNP, now more than fifteen years old, were relatively easy to draft -- and I don't want to trivialize what they did -- because they had to do little more than describe techniques, approaches and strategies for drug development that had already worked, techniques that allowed industry to get drugs on the market.

Plus, writing the guideline was a relatively simple matter of recounting the nature of the patients studied, the types of controls employed, the identity of outcome assessment measures that were sensitive to the drug effects.

Now, to be fair, we don't have a similar situation

with dementia. You can't use this strategy because we don't have a drug, as most of you know, with the arguable exception of Hydergine, that has ever been approved for use as an antidementia agent in the United States.

In short, I would argue there is no body of robust experience in successfully developing an antidementia drug.

To be fair, drugs with antidementia claims are currently under commercial drug development, but it would be premature for the Agency to endorse the strategies undertaken by the sponsors doing these tests. We have high hopes that the sponsors will succeed in their efforts, but, of course, they may not.

In fact, even if they do, as we may learn during the course of today's symposium, not all experts may agree with the particular choices made by the developers of these putative antidementia agents.

Let me give you a concrete example. The majority of recent cholinomimetic drug trials relied upon in enrichment strategy involving the use of a prerandomization cholinergic challenge to identify potential responders; that is, to enrich the likelihood of having a drug-sensitive population. In theory, this maneuver makes a lot of sense.

The problem is, you really have no empirical evidence to convince you that it really does. In fact, you would have to wonder whether or not the possibility exists

that this maneuver could actually be discarding what has become a very valuable commodity; that is, Alzheimer's patients with appropriate entering criteria for selection in the drug trials.

In some, as that is an example of the problem, we believe it is still to early in our collective experience to issue formal rigorously-designed guidelines.

I am aware, of course, that many are concerned that a lack of guidelines has discouraged antidementia drug development because it adds uncertainty to what is already a risky and very expensive undertaking, perhaps. But it is also important to remember that the lack of certainty associated with commercial antidementia drug development is offset by certain potential rewards.

It was of interest to me that a recent pink sheet, a drug agency newsletter, cited a financial investment expert's estimate of the potential domestic sales of antidementia drugs to be in excess of $6 billion, annually. So there is something at the end of the rainbow, at least from the point of view of financial incentives.

Moreover, my colleagues and I have sought to reassure those interested in developing antidementia drugs that the lack of an approved antidementia drug is a reflection of the inadequacies of the drugs so-far tested, not of our assessment methodologies or imagined regulatory biases.

Specifically, if a drug were to exert a clinically-substantive antidementia effect, highly-sensitive rating scales will not be needed to document them.

Moreover, in my own view, even clinically modest antidementia effects should be detectible by skilled clinicians working with global ratings and/or any one of a number of currently available assessment scales, many of which have been described and critiqued, a whole group of them in a very useful edition of Psychopharmacology Bulletin developed by the Aging Branch of the NIMH.

If a sponsor, then, can obtain evidence in adequate and well-controlled investigations to document that a drug has a predictable beneficial effect on one or more of the cardinal signs of Alzheimer's, and I am begging the definition of cardinal signs for the moment, his drug will be likely to gain Agency support for approval provided, of course, that the drug is not unreasonably unsafe relative to the benefits its use confers.

That is the old safety/risk issue.

It is also true, however, that drugs are not approved on the views and judgments of Agency staff alone. What outside experts think, especially experts who are likely to serve on or appear before the Agency's advisory committees, is obviously of critical importance to everyone, the Agency and the regulated industries alike.

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Consequently, especially in the absence of officially-approved guidelines, there is much to be gained by creating an opportunity for those interested in the development of antidementia drugs to learn, first-hand, what is or what is not accepted as "state of the art" in antidementia drug assessment, and to learn it from those who, in effect, decide what is state of the art.

It was with this in mind that we organized this symposium. The symposium, then, is intended to serve as a forum for the open and free exchange of information and ideas among acknowledged experts about the development, testing and assessment of drug products that might be classified as antidementia agents.

I want to emphasize, again, "might" be classified because we have no fixed idea, as yet, what characteristics allow you to classify a drug in this yet-hypothetical class.

Thus, the symposium should be viewed primarily as an educational event. It has no immediate regulatory purpose. Importantly, it is not intended -- and I stress "not" -- to produce a set of official of quasi-official cookbook-like recommendations on how best to develop an antidementia drug.

It is too early to reach that kind of premature closure.

Some official document or guidance may result, but

that is not a necessary or -- how shall I put it -- certain outcome of this meeting.

In any case, it is hoped that the regulator and regulated alike will learn much about the current state of the field from this symposium. In my view, if this symposium does little more than distinguish between areas of agreement and general consensus, and those of disagreement and controversy, it will be all I can hope it can be.

Whatever the outcome, however, I am pretty certain that the meeting is going to be interesting and informative. The faculty is not prominent, alone, but well-informed, intelligent, clever, witty and entertaining. That is why we picked them.

So, without additional delay, let's get on to the first session which addresses the most basis of epistemological issues, just what it is and what kind of information is needed to show that a drug has some antidementia action.

I am going to call upon Dr. Katz, who is Deputy Director of the Division and Chief of our Neurology Drug group, to introduce the session and the first speaker.

Thank you.

DR. KATZ: Thank you, Dr. Leber. I would like to extend my own personal welcome to our entertaining faculty.

SESSION I: THE NEED FOR CONTROLLED TRIALS

DR. KATZ: The sine qua non for the approval of a

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new drug application in the United States, as mandated by the Federal Food, Drug and Cosmetic Act, is that the application must contain -- and this is a quote -- "substantial evidence of effectiveness defined as evidence derived from adequate and well-controlled clinical investigations."

Section 314.126 of Title 21 of the Code of Federal Regulations defines the types of controlled trials that, depending upon the specific circumstances, can be considered adequate and well-controlled. Specifically, the regulations define five clinical controlled trials; those are placebo concurrent control, dose-comparison concurrent control, no-treatment concurrent control, active-treatment concurrent control and historical control.

As can be seen, the regulations provide for a wide range of studies that might be considered by the Agency to be adequate and well-controlled. The type of study or studies that the Agency might consider acceptable will be determined by a number of factors including, but not limited to, the availability of alternative treatments for the condition under study, knowledge of any possible placebo effect in the condition and the nature of the condition under study.

Obviously, the Agency considers the law and regulation sufficiently flexible to permit the appropriate epistemologically-sound trials to be performed in any clinical setting.

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Further, it has been the specific policy of the Agency, to this point, to require that in Alzheimer's disease, acceptable trials are those which are capable, by design, of demonstrating a difference in outcome between the treatment and control group or groups.

Generally speaking, such trials usually take the form of placebo concurrent control studies. However, the nature of the evidence required to establish efficacy in this setting is not necessarily universally accepted. Some feel that the law imposes an arbitrary requirement for control trials that is unnecessary for the evaluation of proposed treatments for patients with Alzheimer's disease.

In today's first panel, our panelists will present their views on the need for controlled trials of proposed drug treatments in patients with dementia. First, and most critically, the appropriateness of the legal requirement for controlled trials to evaluate drug efficacy should be addressed. Why do we, or do we not, need controlled trials? Does the law incorporate accepted scientific standards? Or, in the case of Alzheimer's disease, does it represent an impediment of the development of treatments.

Indeed, if there were no Food, Drug and Cosmetic Act, what would experts declare to be the standards of proof of efficacy of a drug treatment for Alzheimer's patients?

If time permits, I hope that other related questions

can be addressed by the panel; for example, if controlled trials are deemed necessary, what is the appropriate form of such trials? What are the appropriate control groups? Can the form of such trials vary with differing subpopulations of patients with Alzheimer's disease? What, in general, for example, would be an appropriate claim for a drug intended to be useful for this population?

To get us started, we are fortunate to have as our first speaker Dr. Peter Whitehouse of the University Hospitals of Cleveland. Dr. Whitehouse has been instrumental in helping to elucidate some of the basic pathophysiologic mechanisms of Alzheimer's disease, yet he maintains an active interest in clinical trials in this area.

He is uniquely well-qualified to begin this first symposium The Need for Clinical Trials.

Dr. Whitehouse.

DR. WHITEHOUSE: Good morning.

[Slide.]

I want to start my talk this morning with a slide from an article by Dr. John Noseworthy. It is actually the title of an article which appeared in Neurology, the slide kindly provided for my by Elkan Gamzu. The slide, for those of you that can't see it, says, "There are no alternatives to double-blind control trials."

At least in the context of this discussion, which

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as Dr. Katz said, is that we are looking to establish substantial efficacy.

In exploring this particular position with you, I want to examine two senses of what we mean by controlled trials. The first has to do with its role as a scientific instrument to establish efficacy of a drug. The second, and I think these are important issues that the panel ought to address, are the larger impact of controlled studies in a broader societal context, the impact of the study in terms of clinical practice and in terms of society at large.

In examining these two aspects of controlled trials, I actually went back and looked up the origin of the word control. It actually means a contra rotula. Rotula means role. But I couldn't figure out what the word role -- and there were about twenty-five definitions of the word role -- and so what it means to go against the role in terms of the control, I am not sure, based on the origin of word.

So I, then, decided I would invent what I meant by the word role. I think in the first sense of the way I want to look at controlled studies, it is against the roll of the dice; in other words, the study is designed to demonstrate that a drug has an effect that we cannot explain on the basis of some other factor that might include the roll of the dice.

As Dr. Katz said, there are some essential elements to a controlled study in this scientific sense of determining

efficacy that we are all well aware of, and I want to just make some comments about these essential features as I think they relate to Alzheimer's disease.

The first, of course, has to do with the need to have a control or comparison group in the first place. The idea is to establish that a new drug has an effect relative to another condition. As Paul has said, in Alzheimer's disease, we don't have lead drugs that have a major effect on the disease that are currently on the market and, therefore, we are in a situation where, for the most part, we look at studies in which the new drug is compared to placebo.

As Dr. Katz mentioned, there are other things that law allows. Obviously, you can compare drugs at different doses. You can compare different drugs, themselves. You can, also, in the law, as he mentioned, include historical controls as a possibility. There are problems with historical controls, obviously, that you are all familiar with.

In this field, because of the differences in populations that go to one clinic or another, because of the variability in the disease, a theme I will return to later, I don't think, at this stage, historical controls represent a very viable option.

So, really, in terms of a controlled study, we are really talking about a placebo group. There are many questions about placebos in Alzheimer's disease that I think

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the academics among us, and industry, ought to be asking themselves.

There are basic questions about what amount of placebo responsiveness occurs in Alzheimer's patients. I think in the mild stages of the disease, there is probably no reason to believe that placebo responsiveness is any different. But in the later stages of the disease, when the patient cannot remember that they were in the clinic even an hour after their visit, what does that do to the placebo effect? I think there are questions there about the response of the patient.

However, as you are all aware, and as Dr. Leber mentioned, we are also dependent in this field on measurements that we will be hearing about, particularly tomorrow, that are based on the response of the family or clinicians, the global rating scales or the activities of daily living. There is no reason to believe, within this area, that families and physicians are any less responsive to the placebo effects.

I am particularly concerned that families, based on their expectations for positive effects in a drug study, are, in fact, in Alzheimer's disease, particularly prone to interpreting, perhaps, any drug effect, even a mild degree of toxicity, as a positive benefit and affect their more subjective ratings.

So I think that there is work to be done and there are discussions that we can have about the nature of placebos. One aspect of that, I think, pertains to what I just said a minute ago, and that is that families are sensitive to expectations that the drug is going to be effective, has to do with blinding on the possible use of an active placebo.

Obviously, we have come to a point where, as my title slide suggests, double blinding is an intrinsic part of what is considered a well-controlled study.

However, it is rarely asked in studies in Alzheimer's disease, or in other fields, for that matter, whether the individuals were, in fact, blind to the condition or not. A number of the drugs being tried in this area have side effects, dry mouth, GI side effects, which may reveal the treatment condition to the patient, the family or the clinician.

Again, if the families or the clinicians can guess, based on the side effects or some other effect of the drug, that clearly could affect their judgments particularly in the more subjective rating instruments.

The area of active placebo has been actively investigated by Dick Shaber and others in the past in schizophrenia, but it has not been specifically addressed to my satisfaction in the area of Alzheimer's disease.

The third element, now, after a control group and

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double blinding has to do with the issue of randomization. I think, obviously, the point of randomization is to insure that comparable groups are compared, that one does not have a bias in assigning patients to one category of disease or another.

This is a principle that was introduced into clinical trials somewhat later than some of the other principles that I have just mentioned.

I think that we need to give some thought to how we randomize patients in trials, and let me just mention one area that I think bears some further look, and that is the issue of stratification. This relates to something that has already been alluded to in my talk and, I think, by Paul; and that is, there is a tremendous amount of heterogeneity in Alzheimer's disease, and the other dementias, for that matter.

It is not even clear that we are using diagnostic categories that adequately reflect the biology or clinical heterogeneity of the disease. We are still diagnosing Alzheimer's disease the way we did eighty years ago. Is it not unreasonable to think that within what we now call Alzheimer's, there may, in fact, be different kinds of illnesses, different subtypes, that might, in fact, have different responses to therapy.

You hear this kind of an argument after the study

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is done. Somebody will say that a certain percentage of patients responded to this medication. And so we have a subtype of drug-responsive patient. Of course, these post-hoc categorizations are less convincing, but there are clinical and biological reasons to consider that subtypes of patients do exist.

If one has a convincing argument that a mild patient or an early-onset patient might be more responsive to a drug, then one could imagine in the planning a stratification based on those pre-hoc considerations about subtyping.

So I think with regards to control groups, blinding, active placebos, randomization, there are some issues about Alzheimer's disease studies that we can address later in the panel.

I am not going to, this morning, in my talk, address specifically the issue of specific design features such as parallel versus crossover versus mixed designs. I basically will make the weak statement that I also believe that the parallel design is probably, in general, best but that, under appropriate circumstances, given the drugs and the hypotheses, that crossover designs may be appropriate at some point in the development.

Let me make a bridge, now, from my brief comments about the control trials as a scientific enterprise, as a device to establish efficacy for drugs, to the broader

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societal issues that I think a number of you in the audience are interested in.

Let me do that by pointing out that the history of what I have described before in the early part of this talk is old. These principles that I have just enumerated have been described since the 1500's and 1600's. Since we are interested in dementia, let me give you an example of a study that was done and reported in 1799 by a man by the name of Hegarth.

He was evaluating Perkins tractors. These were metal rods that you would pass over the body of somebody. There was a lot of pseudo specificity in this one, Paul, but they were used in part to treat violent cases of insanity which, at that time, probably included patients who were demented.

So here we are in the 1700's with a treatment for dementia. Hegarth compared the metal -- it was all in the metal -- tractors, rods, to wooden rods. Let me just quote from his work: "Let their, the tractors, merit be impartially investigated in order to support their fame, if it be well-founded, or to correct the public opinion if merely formed upon delusion. Such a trial may be accomplished in the most satisfactory manner and ought to be performed without any prejudice.

"Prepare a pair of false, exactly to resemble the

true, tractors. Let the secret be kept inviolable, not only from the patient but every other person. Let the efficacy of both be impartially tried, beginning always with the false tractors."

We might fault him for his design issue here.

"The cases should be accurately stated, and the reports of the effects produced by the true and false tractors be fully given in the words of the patients.

" On January 7, 1799, the wooden tractors were employed. All the five patients, except one, assured us that their pain," he was using it in this situation to treat arthritis, "was relieved. On the following day, the metal rods were used. All the patients were in some measure, but no more, relieved by the second application except one who received no benefit from the first operation and who was not a proper subject for the experiment, having no existing pain but only stiffness of her ankle."

Again, we might argue with the post-hoc removal of subjects from his design, but never the less, if you can read through the language there, you can see a lot of the principles that I have just gone through were present in 1799.

Even back then, there were issues about the impact of those studies on society because, after that study was published, the President of Yale, for a number of years, continued to sell Perkins tractors. So it is clear to all

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of us, even today, that it is not appropriate just to look at a drug trial as solely an instrument that a small group of people participates in to determine efficacy.

We are well aware, as clinicians, members of industry, as members of the regulatory part of this process, that there is something changing about people's attitudes about controlled studies.

It is particularly ironic that as Europe is adopting, at a reasonable rate, some of the standards that we have developed in these countries and that the FDA under Paul's leadership is responding, as this meeting is, to the need for facilitated review in illness in AIDS and Alzheimer's disease, that there is an unsettling presence of concern and criticism in the larger world that we all are members of.

I think we are all aware, for example, of a major New York paper which has called for the selective repeal of the 1962 Kefauver Amendment that, in fact, includes in it the standard that drugs be efficacious. This is selective because they were talking only about AIDS.

But I think if one also reads that paper, there is a pervasive tone that science and FDA are somehow at odds with patients, and that somehow the goals of these two groups have become dissociated. To me, this is absolutely distressing because, in my opinion, the process that we have in this country is the best hope for the development of efficacious

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drugs and not the impediment that, I think, we are hearing about.

So it is this kind of publicity that I think makes me include, at least briefly, in this talk, some concern about where we are going with regards to controlled studies in the larger society. I think, quite frankly, that the problems we are having in this area is, in fact, part of a more general and equally-dangerous antiscience and anticlinical medicine atmosphere in this country.

We in this room are, in part, responsible for creating that. You are seeing that in the Fraud and Science hearings here in Washington. You are seeing it in terms of people's concerns about expense in the health care system. I think we would be foolish if we didn't see our attempts to develop controlled trials in this very important area in this broader context.

I don't have the time to dissect out why this has happened, but I urge you strongly to consider that we need to take certain actions. I think those of us involved in this process, to assure that controlled trials remain as the appropriate vehicle for establishing efficacy and that the results of trials are listened to, need to educate people about the process of drug development.

Our citizens in this country are very uniformed about the process of biology, let alone drug development. I

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comment the actions of the Treatment Committee of the Alzheimer's Association and the Alzheimer's Association, in general, that David and I and a number of other people are involved in. They have had a series of articles in their newsletter that goes out to tens of thousands of people about the whole process of how drugs are developed.

This has to be continued, I think both with industry and Government and academics playing a role.

We have to avoid creating false expectations. The public is expecting the next drug to be the breakthrough that everybody has to have. They are expecting that to the point that when a drug is reported, everybody wants it.

The best protection, then, for our current system, I think, relates to an educated public. I think, also, we have to look inward at our own behavior as well as outward towards what we can do to responding to the need for the public for more education in this area.

We are already a regulated industry, but there is danger that we could become more of a regulated industry. I am particularly concerned about regulations that have to do with promoting the relationships between academics and industry. As professionals -- one of the definitions of professionals, as you all are well aware, is that society assigns a certain amount of self-regulatory behavior to that profession.

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Scientists are supposed to watch themselves. Physicians are supposed to watch themselves. If we don't do that, there is a danger that we will become somewhat depro- professionalized and, in fact, overregulated beyond where we are now.

I think we have to avoid self-serving or institutional-serving publicity that is premature about drugs and their effects in Alzheimer's disease. We have to be aware of the appearance of, or actual conflict of interest, in ways that in this field we have not been, particularly where investigators or their institutions can benefit directly from the outcomes of trials.

As you are aware, in the New England Journal of Medicine, other groups in other areas such as the post- coronory artery bypass lipid-lowering trials that my colleague, Bernadine Healy, has been involved in, have published guidelines for relationships between academics and industry as it relates to these particular trials.

I think we have to give serious consideration to these kinds of issues in this area of scientific enterprise that we are all involved in.

So, in summary, I have tried to communicate to you two senses of contra rotula, if you will; one, some very brief comments that are familiar to many of you in the narrow scientific sense about how we can, in fact, preserve our

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ability to establish that there are no alternatives to double-blind clinical trials.

But very briefly, and though I have done no justice to it, I have given you a sense that there is a broader social environment in which we are all conducting our enterprises and that there is a danger that even scientifically-adequate controlled studies will become uncontrolled in a larger environment having to do with possible problems with publicity or conflict of interest and that even if we in the room agree that this, in fact, statement is true -- and, at one point, I thought about taking a poll, but I decided against that, in the audience -- but even if we believe that this kind of a statement is true, it is our job to convince a larger audience that this remains the best hope for the development of drugs in this area.

Thank you.

DR. KATZ: Thank you, Dr. Whitehouse. I would ask the members of the first panel to come to the front. Thank you.

The initial panel of the symposium will begin now. I don't think that there are too many groundrules here. I think that if anyone has anything to say, they ought to just say it. I don't think that we ought to recognize hands. If no one says anything, I may have to call on people.

I would just lead off with a general question which

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is basically a restatement of the title of this particular symposium, the use of controlled trials, and open it up to the panel for either an endorsement or a difference of opinion with Dr. Whitehouse' contention that there is a need on many levels for controlled trials, there are no alternatives.

After all, this is a condition which is fatal, inexorably progressive, hopeless. There are no available treatments. Let me play devil's advocate for a minute. Why shouldn't people be permitted at this time to avail themselves of any treatment that has any hope associated with it? Do we, in fact, need trials and could you please articulate your reasons for your answer.

DR. DRACHMAN: Someone has to be a devil's advocate here, and I think, perhaps, I will start with a devil's advocate point of view. First of all, I enjoyed your slides, Peter. I wanted to let you know that.

DR. WHITEHOUSE: It was Elkan's slide. It wasn't even my slide.

DR. DRACHMAN: I thought it was very helpful. But the point I would like to make has to do with the difference between the set of rules that are required to establish efficacy and safety and the set of rules that enable us to find drugs that may work.

Our biggest problem, at the moment, isn't necessar-

ily that we can't nail down, using precise measures, the exact degree of efficacy of powerful and effective drugs. We don't have any drugs that are really doing a hell of a lot. That is where I would start.

So I am very concerned that the type of approach that we state -- that includes me, of course -- and the kind of literature that the Alzheimer's Association publishes, as Peter alluded to, in its newsletters, et cetera. The kind of information that we put out gives a very negative scent to those individuals who may need a very open mind in trying out different kinds of drugs.

Let me just allude to an experience I had last week when I talked to a high-level representative of a well-known drug company who explained to me that they had tried a certain drug in rats and it had showed some improvement in maze behavior and in some go/no-go avoidance-type responses. And they had, on the basis of that, designed a trial which was essentially the same as the THA trial; that is, enrich-ment-type studies and make sure that everybody has a Mini-Mental State, Marshal, between 15 and 23, et cetera, so that before anyone had any idea of whether this drug had any effect whatsoever in a single human being, a very carefully controlled 20-center trial was being designed.

If I can think of anything that would constrain investigation, thought, imagination, the discovery of

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intuitive insights into what works and what doesn't work, I would say that starting out with a very careful controlled study would be that.

That is where you end up. That isn't where you start. And that would be the major point I would make.

DR. WHITEHOUSE: David, I wouldn't disagree with that. But, as I said in my comments, we are talking about here in the context of establishing efficacy from a regulatory point of view, so I am not sure we would disagree much.

DR. KATZ: Yes. I would just second what Dr. Whitehouse says that I think we might make a distinction between the drug development, in toto, from Day 1 and meeting the FDNC Act's requirement for substantial evidence of efficacy. At least, I think we might start out discussion the types of trials that are necessary to meet that requirement.

DR. WURTMAN: We are invited to be broad in our discussions. Let me broaden the discussion a bit beyond Alzheimer's disease and end with a question to Dr. Leber and the Food and Drug Administration.

Here we are charged with trying to develop chemicals that are effective in treating a disease that lacks known biological markers. That is the tough part. There is no chemical that we have agreed we can measure in cerebral-spinal fluid or blood. There is no autonomic index. We

have no biologic marker that we can follow in Alzheimer's patients as an index of efficacy.

Also, of course, we have no lead drug that has been shown to affect the patients and to see whether that drug affects the biological markers.

As opposed to certain other behavioral conditions, there are no events that we can count. We can't see whether the number of headaches has decreased, or the numbers of grams of substances that are abused has diminished in Alzheimer's disease.

The rating scales that have been proposed and that are used for Alzheimer's disease do not measure biologically-essential phenomena like how much somebody weighs or how much they eat or how many hours of sleep or how often they have sex and with what success or what have you.

Alzheimer's is not a cyclic phenomenon, as opposed to certain types of depression where, in some ways, the person can serve as his own control. So what we are left with, for the most part, are clinical judgments and rather synthetic scales that sometimes do work.

My question, then, is are there other behavioral diseases that the FDA has now regulated -- who are regulating treatments of, I should say, not the disease. But I am thinking specifically of anxiety or, very recently, obsession, obsessive-compulsive neurosis, other behavioral disorders